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# THE JOURNAL OF HYGIENE

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# CONTENTS.

No. 1 (January).

	PAGE
CASTELLANI, A. Notes on Cases of Fever frequently confounded with Typhoid and Malaria in the Tropics. (Three Charts.) . . .	1
DUDGEON, L. S. and DUNKLEY, E. V. The <i>Micrococcus neoformans</i>	13
HEWLETT, R. T. and BARTON, G. S. The Results of a Chemical, Microscopical and Bacteriological Examination of Samples of London Milks . . . . .	22
MARSHALL, W. E. Note on the Occurrence of Diphtheria Bacilli in Milk . . . . .	32
CURRIE, J. R. On the Supersensitisation of Persons suffering from Diphtheria by Repeated Injections of Horse Serum. (Three Diagrams.) . . . . .	35
CURRIE, J. R. Examples of the Immediate and of the Accelerated Reaction following Two Injections of Anti-diphtherial Serum .	61
LEDINGHAM, J. C. G. On the Relation of the Antitoxin to the Globulin-Content of the Blood Serum during Diphtheria Immunisation. (Four Charts.) . . . . .	65
LEDINGHAM, J. C. G. Notes on the Leucocyte-Reaction during the Immunisation of the Horse and Goat with Diphtheria Toxin .	92
NOON, L. On the Occurrence of Toxic Compounds of Tetanus Toxin and Antitoxin, Tetanus Toxin and Brain Emulsions. (One Figure.) . . . . .	101
BASSETT-SMITH, P. W. The Treatment of Mediterranean Fever by means of Vaccines, with Illustrative Cases. (Seventeen Charts.) .	115
ARKWRIGHT, J. A. On the Occurrence of the <i>Micrococcus catarrhalis</i> in Normal and Catarrhal Noses and its Differentiation from other Gram-negative Cocci . . . . .	145
GREEN, A. B. A Note on the Influence of the Chemical Rays of Daylight on Vaccinia in Animals . . . . .	155
PUBLICATIONS RECEIVED . . . . .	161

---

## Contents

### No. 2 (April).

	PAGE
GREENWOOD, M., Junr. and THOMPSON, T. On Meteorological Factors in the Aetiology of Acute Rheumatism. (Four Diagrams.) . . .	171
MCCRAE, J. and STOCK, P. G. Some Experiments with Fluorescein as an Agent for the Detection of Pollution of Wells. (One Map.) . . .	182
ARKWRIGHT, J. A. On Variations of the Meningococcus and its Differentiation from other Cocci occurring in the Cerebro-Spinal Fluid . . . . .	193
SMITH, J. H. On the Absorption of Antibodies from the Subcutaneous Tissues and Peritoneal Cavity. (Five Figures.) . . . .	205
REVIS, C. and PAYNE, G. A. The Acid Coagulation of Milk. (Curves A, B, C.) . . . . .	216
NUTTALL, G. H. F. and GRAHAM-SMITH, G. S. Canine Piroplasmosis. VI. Studies on the Morphology and Life-History of the Parasite. (Plates I, II, III and Fourteen Diagrams.) . . . .	232
WENYON, C. M. Action of the Colours of Benzidine on Mice Infected with <i>Trypanosoma dimorphon</i> . . . . .	273
IMMS, A. D. On the Larval and Pupal Stages of <i>Anopheles maculipennis</i> , Meigen. (Plates IV and V and One Figure.) . . .	291
IN MEMORIAM. ALLAN MACFADYEN. (Plate VI.) . . . .	319

### No. 3, Extra "Plague Number" (July).

REPORTS ON PLAGUE INVESTIGATIONS IN INDIA,  
issued by the Advisory Committee.

(Continued from Volume VI, p. 536.)

Plates VII to XII.

XI. The diagnosis of natural rat plague. (Plate VII.) . . .	324
XII. The pathological histology of the spleen and liver in spontaneous rat-plague, with observations on the experimental infection. By J. C. G. Ledingham, M.B., B.Sc., M.A. (Plates VIII and IX.) . . .	359
XIII. Transmission of plague by feeding rats with infected material. . . . .	373
XIV. On the significance of the locality of the primary bubo in animals infected with plague in nature . . . . .	382



# Contents

V

	PAGE
XV. Further observations on the transmission of plague by fleas, with special reference to the fate of the plague bacillus in the body of the rat flea ( <i>P. cheopis</i> ) . . . . .	395
XVI. Experimental production of plague epidemics among animals. (One Chart.) ( <i>Second Communication.</i> ) . . . . .	421
XVII. Experiments in plague houses in Bombay. ( <i>Second Communication.</i> ) . . . . .	436
XVIII. On the external anatomy of the Indian rat flea ( <i>P. cheopis</i> ), and its differentiation from some other common fleas. (Plates X to XII.) . . . . .	446
XIX. On the natural occurrence of chronic plague in rats . . .	457
XX. A note on man as a host of the Indian rat flea ( <i>P. cheopis</i> )	472

## No. 4 (July).

SAVAGE, W. G. The Bacteriological Examination of Surface Wells	477
CRAW, J. A. On the Danysz Effect with reference to the Toxin-Antitoxin Reaction. (One Figure.) . . . . .	501
CRAW, J. A. and DEAN, G. On the Estimation of Free Diphtheria Toxin: with reference to the relations existing between lethal doses, lethal times and loss in weight of the guinea-pig. (Two Figures.) . . . . .	512
ARMIT, H. W. The Toxicology of Nickel Carbonyl. (Four Figures.)	525
GRAHAM-SMITH, G. S. A Cystic Disease of the Heart, Gizzard and Muscles of Young Grass Parakeets ( <i>Psittacus undulatus</i> ) due to a Protozoon Parasite. (Plates XIII and XIV.) . . . . .	552
CASTELLANI, A. Experimental Investigations on Framboesia Tropica (Yaws). (Plates XV and XVI and One Figure.) . . . . .	558
TODD, C. Some Experiments on the Filtration of Cattle Plague Blood. (Seven Charts.) . . . . .	570
MARSHALL, W. E. The Para-dimethyl-amido-benzaldehyde Test for Indole. (Three Charts.) . . . . .	581
CRAW, J. A. On Variation in Weight of Normal Guinea-pigs in relation to the Estimation of Free Diphtheria Toxin . . .	589

	PAGE
CUMFSTON, H. A Contribution to the Bacteriology of Post-Scarlatinal Diphtheria . . . . .	593
CUMFSTON, H. The Relative Frequency of Various Types of Streptococci in Scarlatina . . . . .	599
GOODALL, E. W. On the Supersensitisation of Persons by Horse-serum	607

### No. 5 (October).

HOLST, A. Experimental Studies Relating to "Ship-beri-beri" and Scurvy. I. Introduction . . . . .	619
HOLST, A. and FRÖLICH, T. Experimental Studies Relating to "Ship-beri-beri" and Scurvy. II. On the Etiology of Scurvy. (Plates XVII and XVIII.) . . . . .	634
MALDEN, W. Some Observations on the Condition of the Blood in Men Engaged in Anilin Dyeing and the Manufacture of Nitrobenzine and its Compounds. (Two Charts.) . . . .	672
PUBLICATIONS RECEIVED . . . . .	686

### No. 6, Extra "Plague Number" (December).

REPORTS ON PLAGUE INVESTIGATIONS IN INDIA,  
issued by the Advisory Committee.

(*Continued from Volume VII, p. 476.*)

(Plates XIX to XLI with seventy-six maps and charts.)

XXI. Digest of recent observations on the epidemiology of plague .	694
XXII. The epidemiological observations made by the Commission in Bombay City . . . . .	724
XXIII. Observations made in four villages in the neighbourhood of Bombay . . . . .	799
XXIV. General considerations regarding the spread of infection, infectivity of houses, etc. in Bombay City and Island . .	874
XXV. Observations in the Punjab villages of Dhand and Kasel .	895
INDEX OF AUTHORS . . . . .	987
INDEX OF SUBJECTS . . . . .	990

NOTES ON CASES OF FEVER FREQUENTLY CON-  
FOUNDED WITH TYPHOID AND MALARIA IN  
THE TROPICS.

BY ALDO CASTELLANI, M.D.,

*Director of the Clinic for Tropical Diseases, Colombo (Ceylon).*

EVERY practitioner in Tropical Countries knows how frequently cases of fever occur in which it is impossible to arrive at a definite diagnosis.

30 Much light has been thrown on the subject of long-continued fevers by the researches of Leishman, Donovan, Manson, Rogers, etc., especially with regard to Kala-azar. Moreover, the work of Wright, Lamb, and others, has shown that some other forms of long-continued tropical fevers represent cases of Malta fever.

Tropical Fevers of short duration have been recently investigated by Rogers, who has defined a very interesting influenza-like type which he names "seven days' fever."

I desire to call attention to yet other forms of fever lasting two to three weeks, or at times much longer, and characterized by the following symptoms: Temperature generally irregular; pulse frequently very slow; spleen not sensibly enlarged; no roseola; slight intestinal symptoms occasionally present; Widal test constantly negative; malarial parasites absent. Such cases, in Ceylon at least, are of frequent occurrence and are generally diagnosed as malaria, or typhoid, or, since I demonstrated the presence of the latter disease in the Island, as paratyphoid<sup>1</sup>.

While admitting that typhoid fever, especially in tropical climates, may run a most atypical clinical course, I am convinced that a certain proportion of the cases referred to are neither typhoid nor paratyphoid,

<sup>1</sup> Four cases of paratyphoid have come under my observation in Ceylon: three were due to *B. paratyphosus* A and one to *B. paratyphosus* B.

and certainly not malaria, though occasionally a malaria infection may be present at the same time.

I give very briefly the history of four such cases—in which a bacteriological investigation was made.

CASE 1. Austrian gentleman, 23 years of age. Was in Ceylon for two months (January and February, 1905), visiting various places in the interior. On returning to Colombo from the hills he began to feel unwell while in the train, experiencing severe frontal headache and slight rheumatoid pains all over the body. He had no shivering, and no intestinal symptoms. In the evening he took his temperature and found it to be  $102.4^{\circ}$ . Believing that he was suffering from malaria he swallowed a large dose of quinine (20 grs.) which caused very severe ringing of the ears. In the morning the temperature was much lower ( $99.4^{\circ}$ ) and the patient, after having taken five grains more of quinine, drove to Mount Lavinia, a place near Colombo. Coming back in the afternoon he complained again of great malaise and headache; he again took quinine—10 grains. When he asked me the same evening

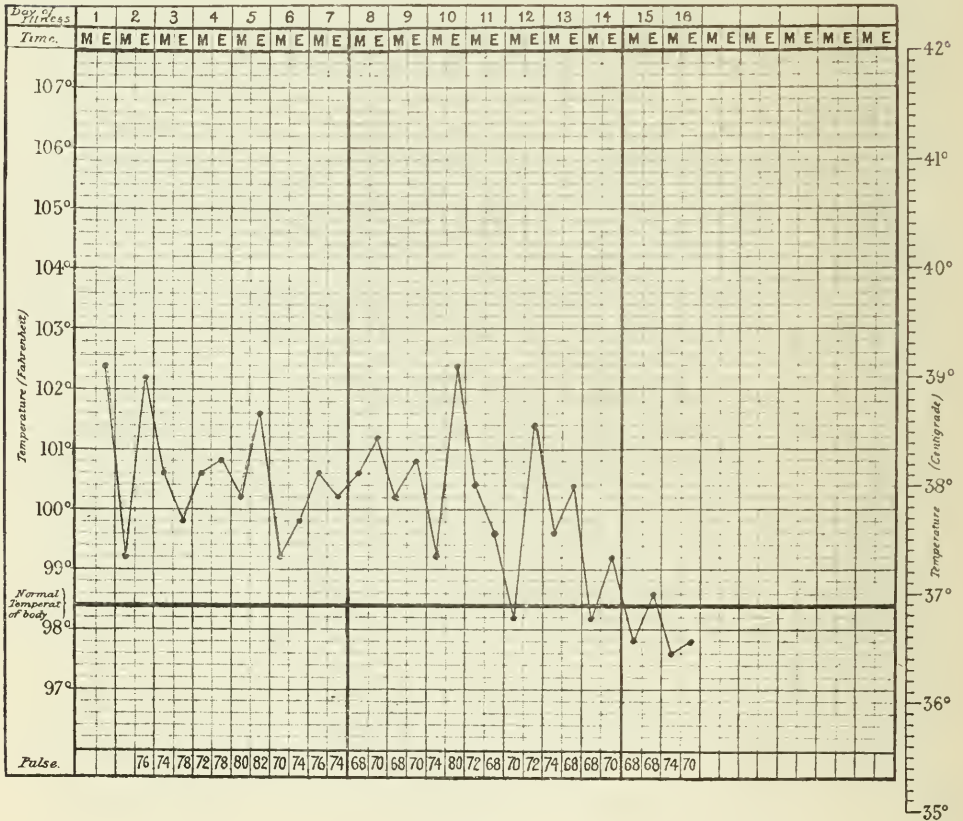


Chart 1.



to examine him his temperature was  $102.2^{\circ}$ , pulse 76. The tongue was slightly furred; the spleen could not be felt; and no roseola spots or any other rash were seen. There was no diarrhoea and the analysis of urine was negative. The fever continued for 12 days longer and was very irregular in its course as shown by temperature chart No 1. The pulse always remained slow; this want of correspondence between pulse-rate and temperature was observed by me in several other similar cases, though it cannot be said to be a constant symptom. The spleen could never be palpated; roseolae did not appear; Widal was negative. As regards intestinal symptoms, there was nothing to be noted with the exception of a tendency to constipation which, however, was never severe. The patient never complained of serious subjective symptoms and the headache left him after the first few days; his mind always remained clear. He got gradually better and convalescence was rapid.

It may be noted that during convalescence, and also afterwards, the pulse-rate remained about the same viz: 70 to 74 per minute.

CASE 2. Strongly-built man, 50 years of age—a retired officer from the German Army. No previous disease of any kind. Began to feel ill while on board a

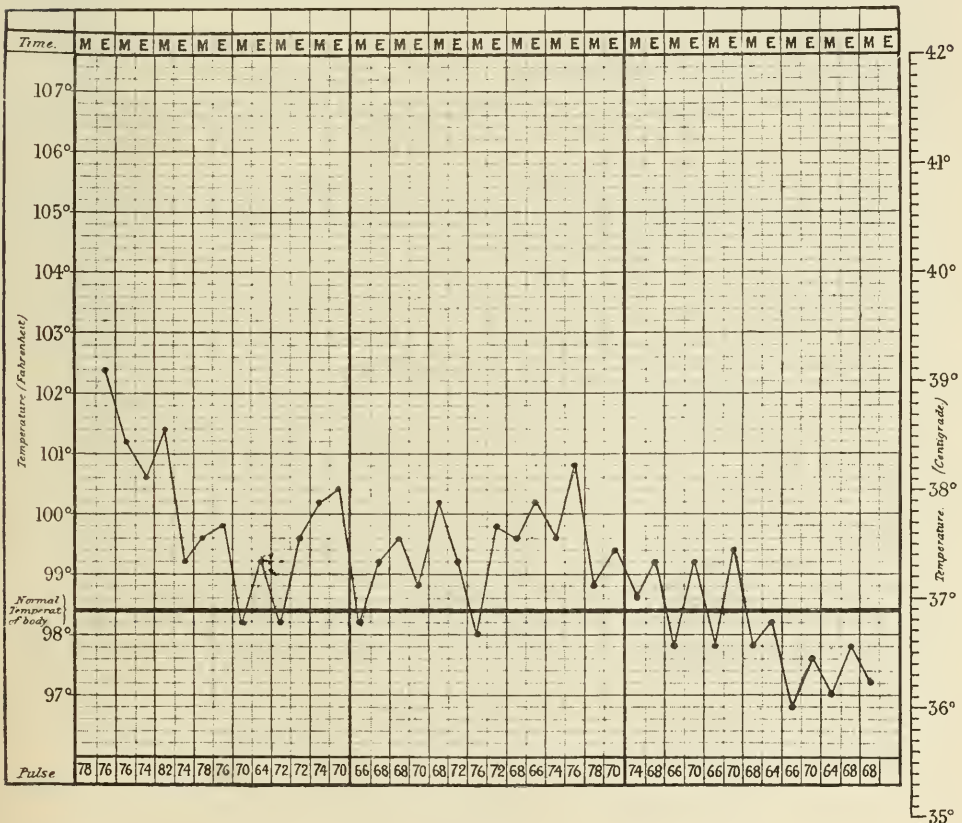


Chart 2.

German steamer during the voyage from Aden to Colombo with malaise, continuous headache, rheumatoid pains all over the body, irregular rises of temperature, slight diarrhoea. He never felt so ill as to have to remain in his cabin. As there was a case of enteric on board, the same disease was suspected in this patient and he was accordingly advised to land at Colombo. During the first three days after landing in Colombo he felt quite well and was getting ready to go up country when suddenly, in the afternoon, his temperature rose to  $103.2^{\circ}$ . There was slight shivering, and severe headache, diarrhoea becoming a prominent symptom, ten or twelve liquid motions being passed in a few hours. I saw the patient in the evening: temperature  $102.4^{\circ}$ , pulse 78; no roseola or any other rash; examination of the chest negative; spleen not enlarged; no pain on palpation of abdomen; the stools which were liquid and yellowish did not contain blood or muco-pus. The fever lasted sixteen days longer and its course was very irregular as shown by temperature chart No. 2. The pulse always remained slow. The spleen could never be palpated and roseola spots never appeared. The diarrhoea lasted for three days only, and the stools never contained blood. The analysis of the urine was negative. Excepting during the first four days the patient did not complain of headache or any serious subjective symptom.

Treatment consisted in the exhibition of the usual intestinal antiseptics and in keeping the patient on liquid diet.

CASE 3. An English civilian, 32 years of age, who had been in the island many years. No previous disease of importance with the exception of two slight attacks of malaria. In the first week of June 1904 he began to feel unwell with lassitude, loss of appetite, and at times feverishness. Being very anxious to go on working, he refused to rest and continued to attend to his duties. When I examined him some days later his temperature was  $103.4^{\circ}$ ; pulse 74 (his pulse rate in health varies from 68 to 72). Spleen could not be felt; no roseola or other rash present. No intestinal symptoms; analysis of urine negative. The examination of the blood showed a few parasites of benign tertian. Quinine was given in large doses for three consecutive days. The malaria parasites disappeared from the peripheral circulation but the temperature was very little affected. The patient was therefore sent to the general hospital. The fever lasted nearly three weeks, presenting a very irregular type. No roseola appeared and the spleen could never be palpated; no diarrhoea. Widal test always negative. During the last days of the illness the inguinal glands, particularly those of the left side, became swollen and slightly painful. This adenitis lasted for several days. The patient gradually improved and shortly afterwards left the hospital. Being fond of physical exercise he did much rowing and fencing. He soon noticed again (about three weeks after having left the hospital) an enlargement of the inguinal glands of the left side, one of which showed signs of suppuration. An incision was made in the gland and the pus collected in a sterile Petri dish with all aseptic precautions. From the pus a germ, to be described presently, was grown in pure culture. Several other inguinal glands became enlarged but they did not suppurate; with rest and local iodine applications the glandular swelling slowly subsided.

CASE 4. Austrian medical man, 24 years of age, arrived from China in April 1905. A week before reaching Colombo he began to feel ill with lassitude, headache,

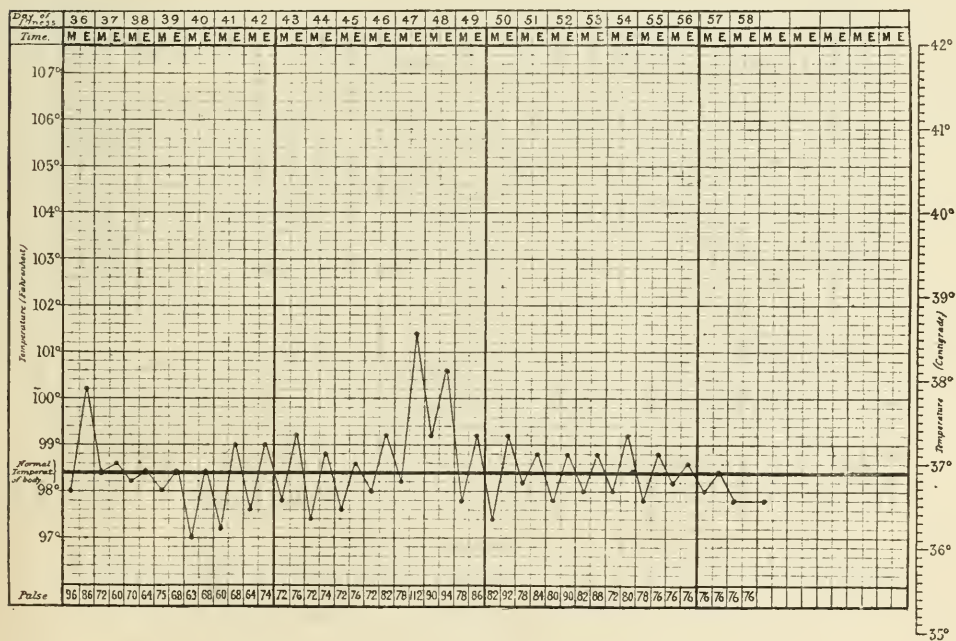
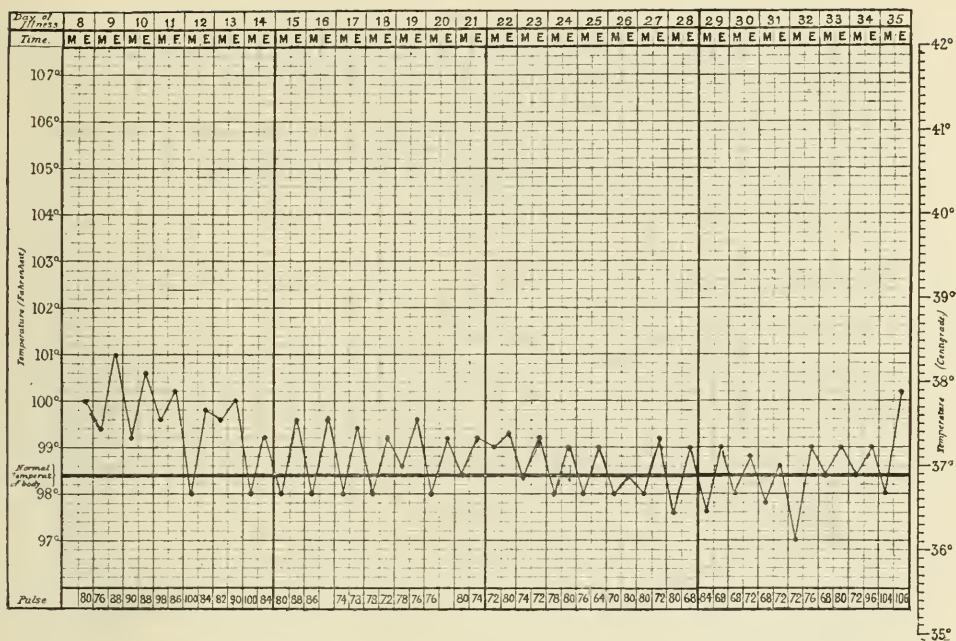


Chart 3.



diarrhoea (10 to 15 motions daily without muco-pus or blood), irregular fever. The patient was landed at Colombo and entered a private nursing home. The disease lasted about two months. The course of the temperature is shown in Chart 3, the diarrhoea ceased very soon and was followed by a long period of constipation. The tongue was generally very little furred; the spleen was very slightly enlarged at the end of the second week; roseola spots were never seen. The convalescence was very prolonged, the patient suffering repeatedly from attacks of nervous tachycardia, a condition to which he had been subject for several years before this illness.

*Microscopical and Bacteriological Examination of the foregoing cases.*

*Case 1.* Malaria parasites constantly absent, even several days after the quinine administration had been discontinued. The relative number of large mononuclear leucocytes was not increased, the count being large mononuclear 8%; polymorphonuclear 65%; small mononuclear 20%; transition forms 5%; eosinophiles 2%. Number of leucocytes per c.mm. 11,000.

The Widal test was negative; it was applied five times, three times during the course of the disease and twice during convalescence—the dilutions of the blood being 1 in 60; 1 in 40; and 1 in 20; time of observation 2 hours.

The blood was tested with two strains of *B. paratyphosus* A; one strain of *B. paratyphosus* B; and three strains of the paradysentery bacillus: none of these germs were agglutinated.

Having obtained the patient's permission on the 5th day of the disease, I drew a few cubic centimetres of blood from an arm-vein by means of a sterile syringe, using the ordinary aseptic precautions. The blood was inoculated at once into several large flasks containing each 300 c.c. of ordinary beef broth according to the "dilution method" introduced by me for typhoid<sup>1</sup>. The flasks were inoculated at 37° C. After two days, three out of six flasks showed a general turbidity, due to the growth in pure culture of a germ to be described below.

The microscopical examination of the stools of the patient did not show anything worth noting with the exception of a very few eggs of *Tricocephalus dispar*. The stools were investigated bacteriologically, using Drigalsky's medium; germs of the coli group only were found. The urine, twice examined bacteriologically, proved sterile.

*Case 2.* The blood was examined for malaria six times; malarial parasites and pigment constantly absent. Number of large mononuclear leucocytes 12%; polymorphonuclear 62%; small mononuclear 18%;

<sup>1</sup> See *Settimana Medica*, 1899, No. 3; *Riforma Medica*, 1900, Nos. 8 and 9.



eosinophiles 8%. Widal, repeated six times, negative. Serum reaction using various paratyphoid and paradysentery strains also negative. The patient having no objection, a few c.c. of blood were taken from a vein and examined as in case 1. In one of the six flasks the medium became turbid after 24 hours and showed the presence of a bacillus identical with that found in case 1. Some colonies of the same germ could be grown from the stools plated, using MacConkey's medium. The urine was sterile.

*Case 3.* The blood, as stated in the short history given above, showed a few benign tertian parasites which soon disappeared after the administration of quinine; the fever however continued its course, showing that it was not due solely to the malarial infection. Widal test repeated many times during the course of the disease, during convalescence, and afterwards, always gave negative results. Serum tests using various strains of paratyphoid bacilli also gave negative results.

The pus from the inguinal gland which had been collected aseptically was plated. All the plates after 24 to 36 hours showed colonies of one germ only which was identical with that cultivated from the blood of cases 1 and 3. Part of the pus was inoculated subcutaneously into a guinea-pig, which remained healthy<sup>1</sup>.

*Case 4.* Malaria parasites or pigment were never found. Plate cultures from the stools yielded many colonies of a germ different to that found in cases 1, 2, and 3. The germ encountered in the stools was also found during convalescence in the urine on two occasions. The blood was not examined for the presence of bacteria, but it was found to agglutinate the bacillus grown from the stools and urine, using a dilution of 1 in 300.

<sup>1</sup> *Note.* Since this paper was written—some months ago—a patient presenting symptoms similar to case 3 has come under my observation. This patient also, after several days of irregular fever, developed an inguinal adenitis, which came to suppuration. From the pus the *B. ceylanensis* A. was grown. These cases cannot, however, in my opinion have anything to do with so-called climatic bubos, which as a rule do not suppurate.

*Description of "Bacillus ceylanensis A" found in cases 1, 2, and 3.*

A rather short rod 2 to 4  $\mu$  in length, resembling closely the typhoid and dysentery bacillus. It is easily stained by the ordinary aniline dyes, but not by Gram. It is non-motile and I have never been able to demonstrate flagella, using various methods of flagella-staining. Brownian movement not very marked.

*Cultural characters.* *Broth*:—Growth fairly abundant with general turbidity of the medium. After two to four days a pellicle is noticeable on the surface of the medium. The pellicle is more or less marked according to the composition of the medium. In the broth as made up in the Colombo Laboratory<sup>1</sup>, the pellicle appears generally after 36 hours' incubation at 37° C. and is well marked. After a few days a certain amount of sediment is present. Gas bubbles are never observed.

*Peptone Water*:—Diffuse cloudiness, no formation of pellicle.

After some days some whitish sediment is present.

*Gelatin*:—No liquefaction. Colonies roundish, delicate, closely resembling the colonies of the dysentery bacillus (Kruse-Shiga type).

*Agar*:—Growth very similar to that of the typhoid or Kruse-Shiga bacillus. Surface colonies roundish and very delicate; much more delicate than those of many strains of coli.

*Action on the various sugar media*:—

*Saccharose*:—No production of acid; no gas.

*Glucose*:—Slight production of acid; no gas.

*Mannite*:—No formation of acid or gas.

*Dulcite, Maltose, Lactose*:—No formation of acid; no gas.

*Milk*:—Slowly acidified and clotted.

*MacConkey's neutral-red agar*:—Colonies roundish, delicate, do not take up the red stain.

*Serum, Serum-agar, Blood-agar*:—The growth in these media does not show anything characteristic.

*Indol formation*:—None.

*Pathogenicity*:—The intra-peritoneal inoculation of one c.c. of broth culture kills guinea-pigs in 24 to 36 hours. The subcutaneous inoculation of ordinary doses does not cause any symptoms in rabbits and guinea-pigs.

<sup>1</sup> Lemco 5 gr., Witte's Peptone 10 gr., Salt 5 gr., Water 1000 c.c.

*Description of "Bacillus ceylanensis B" found in case 4.*

A short non-motile bacillus 2 to 4 $\mu$  in length, easily stained by the usual aniline dyes, not stained by Gram.

*Cultural Characters. Broth*:—Abundant growth; diffuse turbidity; after 4 to 6 days a pellicle is present in the Colombo Laboratory broth. Very little sediment. No gas bubbles.

*Peptone Water*:—Diffuse turbidity, no pellicle, no gas bubbles, very slight amount of sediment after some days.

*Gelatin*:—No liquefaction. Colonies not so delicate as those of the germ found in cases 1, 2, and 3.

*Agar*:—Rounded whitish colonies. Growth very abundant.

*Action on the various sugars*:—

*Saccharose*:—Acid formed, but no gas.

*Glucose*:—Acid, no gas.

*Mannite, Dulcite, Maltose, Lactose*:—Acid formed, but no gas.

*Milk*:—The medium is quickly acidified and clotted.

*MacConkey's neutral-red agar*:—The germ grows abundantly, the colonies taking up a deep red colour.

*Serum, Serum-agar, Blood-agar*:—Growth not characteristic.

*Pathogenicity*:—Intra-peritoneal injection of 1 c.c. of broth culture kills guinea-pigs and rabbits in 20 to 36 hours. Subcutaneous inoculations of 2 to 5 c.c. does not kill guinea-pigs or rabbits.

*Agglutination Reactions of the Strains isolated in cases 1, 2, and 3, and of the Strain isolated in case 4.*

The strain isolated in case 1 was not agglutinated at first (7th day of the disease) by the blood of the patient. Agglutinins however appeared on the 10th day and gradually increased in amount during convalescence, the agglutination limit being dilution 1 in 500.

This germ was tested later on with the blood of cases 2 and 4. It was easily agglutinated by the blood of case 2, using a dilution of 1 in 400, but was not influenced in the least by the blood of case 4.

The strain isolated from case 2 was agglutinated by the blood of the patient, from whom it was grown, using a dilution of 1 in 500. It was not tested with the blood of the other patients.

The strain isolated from case 3 was agglutinated by the blood of this patient (dilution 4 in 300) and also by the blood of cases 1 and 2, (dilution 1 in 400), but not by the blood of case 4.

The strain grown from case 4 was agglutinated by the blood of case 4 (dilution 1 in 300). It was also tested with the blood of case 2 but with negative results.

In the following table are collected the agglutination reactions of the organisms isolated from the four cases, with sera derived from rabbits inoculated with them.

Serum	Agglutination limits with			
	Strain 1	Strain 2	Strain 3	Strain 4
Strain 1	2000	1500	2000	0
Strain 2	4000	4000	3500	50
Strain 3	5000	4000	5000	50
Strain 4	0	0	0	2500

These agglutination reactions, and the cultural characters already described, show that the four strains may be divided into two groups: the strains isolated from cases 1, 2, and 3, on the one hand and the strain isolated from case 4 on the other. The strains grown from the first three cases represent one and the same organism; not so the strain obtained from case 4. For convenience I shall indicate the germ found in cases 1, 2, and 3 by the name of *B. ceylanensis* A and the germ grown from case 4 with the name *B. ceylanensis* B.

#### *Relation of the Organisms Described to other Bacteria.*

The following table, largely based on data regarding paratyphoid and allied organisms given by Morgan<sup>1</sup> and Boycott<sup>2</sup>, will show the principal differential characters of various intestinal bacteria and of the organisms observed by me.

From this table it is seen that the two strains differ from the other intestinal bacteria. They differ also from one another in several important respects, *B. ceylanensis* A producing acid only in glucose, while *B. ceylanensis* B produces acid in all sugars tried; besides *B. ceylanensis* A, does not form indol while *B. ceylanensis* B does. In fact, *B. ceylanensis* B is much more closely related to other intestinal bacteria (*B. pyogenes foetidus*) than to *B. ceylanensis* A.

<sup>1</sup> Morgan (1905). *Brit. Med. Journ.* vol. i, p. 1257.

<sup>2</sup> Boycott (1906). *Journ. of Hygiene*, vol. vi, p. 33.

Bacillus	Motility	Broth	Glucose	Mannite	Lactose	Saccha- rose	Dulcitol	Litmus milk	Indol
<i>Typhosus</i>	+	G.T.	Ac.	Ac.	Nil	Nil	Nil	Ac.	—
<i>Dysentery</i> (Kruse-Shiga)	—	G.T.	Ac.	Nil	Nil	Nil	Nil	Ac. then slightly alkaline	—
<i>Dysentery</i> (Flexner)	—	G.T.	Ac.	Ac.	Nil	Nil	Nil	Ac. then alkaline	+
<i>Enteritidis</i> (Gaertner) Strain I	+	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac. then alkaline	—
<i>Enteritidis</i> (Gaertner) Strain II	—	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac. then alkaline	—
<i>Hog cholera</i> (Evans)	+	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac. then alkaline or trace	—
<i>Coli</i>	+	G.T.	Ac.G.	Ac.G.	Ac.G.	Nil	Ac.G.	Ac.C.	+
<i>Acidi lactici</i> (Hüppe)	—	G.T.	Ac.G.	Ac.G.	Ac.G.	Nil	Nil	Ac.C.	+
<i>Lact. aërogenes</i>	—	G.T.P.	Ac.G.	Ac.G.	Ac.G.	Ac.G.	Nil	Ac.C.	—
<i>Pyogenes foetidus</i>	—	G.T.	Ac.	Ac.	Ac.	Ac.	Ac.	Ac.C.	+
<i>Paracolon</i> "Mair"	+	G.T.	Ac.	Ac.G.	Ac.	Nil	Nil	Ac.C.	+
<i>Paracolon</i> "Day"	+	G.T.P.	Ac.G.	Ac.G.	Nil	Nil	Nil	Ac. then alkaline	—
<i>Valérie 25</i> (Boycott)	+	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac.	+
<i>Valérie 21</i> (Boycott)	+	G.T.	Ac.G.	Ac.G.	Nil	Ac.G.	Ac.G.	Ac.C.	+
<i>Paratyphoid A</i>	+	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac.	+
<i>Paratyphoid B</i>	+	G.T.	Ac.G.	Ac.G.	Nil	Nil	Ac.G.	Ac. then alkaline	slight
<i>Ceylanensis A</i>	—	G.T.P.	Ac.	Nil	Nil	Nil	Nil	Ac.C.	—
<i>Ceylanensis B</i>	—	G.T.P.	Ac.	Ac.	Ac.	Ac.	Ac.	Ac.C.	+

Ac. = acid.

Ac.C. = acid and clot.

Ac.G. = acid and gas.

G.T. = general turbidity.

G.T.P. = general turbidity and presence of pellicle.

The sign + in the motility column indicates that the bacillus is motile; the sign — that it is not motile.

The sign + in the indol column means that there is formation of indol; the sign — that there is not formation of indol.

### Conclusions.

1. There are cases of unclassified fever occurring in Ceylon which on superficial examination may be taken for atypical forms of typhoid, paratyphoid, or malaria.

2. Four such cases have been examined by me bacteriologically. Of these the fourth case showed clinical symptoms somewhat different from the first three cases and the disease lasted longer.

3. From the first three cases a bacillus was grown (in two cases from the blood). The bacillus was non-motile, it produced a pellicle in



broth, acidified and coagulated milk slowly, produced acid but no gas in glucose, and produced neither gas nor acid in saccharose, mannite, dulcitol, lactose; no indol formation. The germ was agglutinated by the blood of the patients.

4. From the fourth case a bacillus was isolated which was non-motile. The bacillus produced a pellicle in broth, acidified and clotted milk quickly, produced acid but no gas in saccharose, glucose, mannite, dulcitol, lactose; it formed indol. The germ was agglutinated by the blood of the patient from whom it was recovered.

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## THE MICROCOCCUS NEOFORMANS.

ITS CULTURAL CHARACTERS AND PATHOGENICITY AND THE RESULTS OF THE ESTIMATION OF THE OPSONIC AND AGGLUTINATIVE PROPERTIES OF THE SERUM OF PATIENTS SUFFERING FROM MALIGNANT DISEASE ON THIS ORGANISM AND ON THE STAPHYLOCOCCUS ALBUS.

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OWING to the somewhat contradictory results which have been obtained by various investigators as to the nature of the *Micrococcus neoformans* and the relation which this organism bears to malignant disease, we have thought it advisable to place on record our observations in a large number of cases of carcinomata, sarcomata and certain cases of anaemia which are known to run a fatal course. Doyen (1886), in a preliminary note communicated to the Academy of Sciences of Paris, stated that he had found small spherical bodies in malignant and other growths which he regarded as micro-organisms. In 1902, at the Surgical Congress, at Berlin, he stated further that he had isolated a micro-organism from simple and malignant growths which, when inoculated into animals, gave rise to neoplastic formations. To this organism he gave the name *M. neoformans*. In his opinion new growths resulted from an infection of the body with this organism. Since then he has published a number of observations showing the beneficial effects which may be obtained by the employment of a vaccine or serum against the *M. neoformans* in malignant disease.

TABLE I.  
*Cultural characters of the Micrococcus neoformans.*

Obtained from two cases of squamous-celled carcinoma and one case of osseous sarcoma, and sent to us through the kindness of Dr Doyen.

Gram	A				B				C				D			
	+				Positive				Positive				Positive			
Agar slope	Typical white glistening growth				Typical white glistening growth				Typical white glistening growth				Typical white glistening growth			
Jelly slope	Liquefied in 4 days				Liquefied in 3 days				Liquefied in 3 days				Liquefied in 3 days			
Neutral-red broth (anaerobic)	No green fluorescence				No green fluorescence				No green fluorescence				No green fluorescence			
Litmus dextrose	Acid +				Acid +				Acid +				Acid +			
Litmus lactose	Acid +				Acid +				Acid +				Acid +			
Litmus saccharose	Acid +				Acid +				Acid +				Acid +			
Litmus maltose	Acid				Acid				Acid				Acid			
Litmus arabinose	Acid				Acid				Acid				Acid			
Litmus raffinose	Unaffected				Acid				Acid				Acid			
Litmus xylose	Acid				Acid				Acid				Acid			
Litmus glycerine	Acid				Acid				Acid				Acid			
Litmus erythrite	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus mannite	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus salicin	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus sorbit	Acid				Acid				Acid				Acid			
Litmus milk	Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change			
Lead acetate	Black precipitate				Black precipitate				Black precipitate				Black precipitate			
Broth	General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci			
Nature of organism	Culture(A) from squamous-celled carcinoma, sent us iv. 1906, by Dr Doyen				Culture(B) from squamous-celled carcinoma, sent us x. 1906, by Dr Doyen				Culture(C) from a sarcoma, sent us x. 1906, by Dr Doyen				Culture (D) the same as culture (A) but grown on artificial media for several months, then inoculated intraperitoneally into a guinea-pig and recovered from the spleen			



Drs Paine and Morgan failed to obtain any improvement by injection of either vaccine or serum in nine cases of malignant disease. Lately, however, the vaccine has been given in carefully regulated doses at definite intervals determined by the opsonic power of the patients' serum with the result that some observers claim to have obtained remarkable results; almost as great an improvement has occurred as was *originally* noticed by Doyen. We do not intend, however, in this paper to discuss the treatment of malignant disease with either a vaccine or a serum against the *M. neoformans*<sup>1</sup>, but only to refer to those points which are of importance in determining the nature of this micrococcus, and its relation to other micrococci of the albus series.

The most striking feature in the above table is the remarkable similarity of the three strains of the *M. neoformans* which we received from Dr Doyen. The results are almost identical in each case although so many media were employed. The first culture which we examined was subcultured for some months on artificial media, and then passed through a guinea-pig, yet, when recovered from the spleen of the animal, the cultural characters were practically identical with those of the original coccus and also with two strains obtained from two other cases of malignant growths. It is difficult to understand how this organism was recognised from other white micrococci by those who have previously studied its cultural characters, as there is nothing in the morphology, staining properties, or in the appearance of the growth on the ordinary laboratory media by which it could be identified. On the other hand, Dr Doyen has sent us an organism obtained from three distinct cases of malignant disease, the three strains of which agree in almost every particular. It occurred to us that if we employed an elaborate series of tests based on the valuable work of Mervyn Gordon, that we should be able to show that this organism might really represent various strains of the *Staphylococcus albus*, but our investigation gives no support to this view. One of us (L. S. D.) has examined large numbers of strains of staphylococci obtained from every possible source and has occasionally met with staphylococci which are identical in their appearance with the *M. neoformans*. These results will be published in detail at some future date.

The *Staphylococcus pyogenes albus* (obtained from Král), which Dr Gordon examined by his ten tests, gave identical results to those which we have obtained with the *M. neoformans*, except that it acidified mannite, but possibly this may be an important difference. It is

<sup>1</sup> This will be dealt with at a later date.

unnecessary in this communication to refer more fully to these points beyond mentioning that the morphology and cultural properties of this organism appear to be constant and that a similar organism is only occasionally met with in simple inflammatory affections.

### *Agglutination Reactions.*

TABLE II.

*To illustrate the number of cases in which Agglutination occurred with the Patients' serum and with the M. neoformans and Staph. albus.*

Total number of cases tested=67.

Operative or ulcerative cases=47.

Non-operative or non-ulcerative cases=20.

The serum of 22	ulcerative	cases agglutinated	<i>M. neoformans</i> .
„ „ 37	„ „	„	<i>Staph. albus</i> .
„ „ 12	non-ulcerative	„	<i>M. neoformans</i> .
„ „ 14	„ „	„	<i>Staph. albus</i> .

N.B. The word ulceration is used merely as a convenient term to denote either an open operation wound or an ulcerating surface formed by the new growth.

### *Nature of the Agglutination Reaction.*

With *M. neoformans* :—

		Serum dilution
25 cases gave a reaction with	1 : 50.	
7 „ „ „	{ 1 : 50, and 1 : 100.	
2 „ „ „	{ 1 : 50, 1 : 100, and 1 : 500.	

With *Staph. albus* :—

36 cases gave a reaction with	1 : 50.	
10 „ „ „	{ 1 : 50, and 1 : 100.	
5 „ „ „	{ 1 : 50, 1 : 100, and 1 : 500.	

In 80 cases *no* agglutination reaction occurred with either organism :

- 38 ulcerative cases of carcinoma.
- 2 ulcerative sarcomatous cases.
- 22 non-ulcerative cases of carcinoma.
- 3 non-ulcerative cases of sarcoma.
- 4 cases of primary anaemia.
- 11 various cases.

Both the microscopical and macroscopical methods were employed for the determination of the agglutination reaction.

In every instance young agar cultures were used about twenty-four hours old, and emulsions were made with sterile normal saline. It was only occasionally that a positive reaction was observed, such as is seen in the case of typhoid fever.

It will be seen from studying the results of the agglutination reaction that the serum of patients suffering from all forms of malignant disease reacts more frequently to a standard laboratory culture of the *Staphylococcus albus*<sup>1</sup> than to the *M. neoformans*, and that by far the majority of cases only react with a dilution of 1 in 50. It was only in exceptional circumstances that a reaction was obtained with a dilution of 1 in 500.

These observations were made with great care and were carefully controlled. Another point of equal importance is that the agglutination reaction is much more frequently met with in those instances in which the new growth had undergone ulceration than in the non-ulcerative cases, and here also a positive reaction occurred more frequently with the *Staph. albus* than with the *M. neoformans*.

In well over half the cases no agglutination reaction occurred (i.e. with a dilution of 1 in 50) with either organism, while of these cases exactly half the number were examples of ulcerative growths. Therefore, in many cases of malignant disease there is complete absence of any agglutination reaction with the high dilutions which we employed in this investigation.

#### *Opsonic Actions.*

It is important to draw attention to the fact that 19 out of the 25 cases of carcinoma, of which the *M. neoformans* opsonic index was determined, had undergone ulceration, and 17 of the 23 cases of carcinoma of which the *Staphylococcus albus* index was made presented similar changes.

Drs Bulloch and Western have shown<sup>2</sup> that there are specific opsonins present in both normal and immune sera. The contact of normal serum with the *Staphylococcus aureus* leaves the opsonic action of the serum for *B. pyocyaneus* unchanged, while the specific opsonins for the *Staph. aureus* were practically removed. We undertook some experiments, therefore, for the purpose of determining whether there

<sup>1</sup> This culture was isolated from the blood during life from a case of acute endocarditis. The same culture was used throughout this investigation.

<sup>2</sup> Bulloch, W., and Western, G. T. "The Specificity of the Opsonic Substances in the Blood Serum," *Proc. Roy. Soc. B.* LXXVII. 1906.

was a specific opsonin in immune sera for the micrococci referred to in this paper.

TABLE III.

*Showing the Opsonic Index in 23 cases of Carcinomata, 4 of Sarcomata, and in various Diseases, also in 5 healthy medical men.*

No. of case		Opsonic Index	
		<i>M. neoformans</i>	<i>Staph. albus</i>
1.	Ulcerating carcinoma of tongue	0·4	—
2.	Carcinoma of liver	0·5	—
3.	„ pancreas	0·2	1
4.	Ulcerating carcinoma of glands of neck	0·65	0·5
5.	„ „ colon	0·78	0·9
6.	Suppurating carcinoma of ovary	0·57	1·1
7.	„ „ penis	0·9	0·9
8.	Carcinoma of pancreas	0·8	1·1
9.	Ulcerating carcinoma of cervix	1·1	1
10.	„ „ glands of neck	0·8	1
11.	„ „ penis	1·1	1·2
12.	„ „ breast	0·7	1·4
13.	Carcinoma of breast	0·6	1·2
14.	Recurrent carcinoma of breast	1·2	1·1
15.	Ulcerating carcinoma of cervix	0·7	1·2
16.	„ „ rectum	1	0·9
17.	„ „ lip	0·5	1
18.	„ „ oesophagus	1	1·1
19.	„ „ lip	0·8	0·9
20.	Duct carcinoma of breast	0·9	1
21.	Ulcerating carcinoma of oesophagus	0·6	0·7
22.	„ „ cervix	0·5	0·8
23.	„ „ colon	0·8	0·9
24.	Malignant disease of peritoneum (?)	1	0·5
25.	Ulcerating carcinoma of oesophagus	0·9	0·8
26.	Sarcoma of scapula	0·6	1·2
27.	Ulcerating sarcoma of tonsil	0·9	0·7
28.	Suppurating sarcoma of parotid gland	0·9	0·9
29.	Sarcoma of meninges (operation)	1	0·9
30.	Pernicious anaemia	0·5	—
31.	Myelaemia	0·9	0·9
32.	„	1	0·4
33.	Adenoma of buttock	1	0·9
34.	A. Healthy medical man	0·9	0·8
35.	B. „ „	1	0·9
36.	C. „ „	0·9	0·75
37.	D. „ „	1·1	0·9
38.	E. „ „	0·4	0·9

*Average opsonic index for each group in the foregoing table.*

With *Staph. albus* :—

	Opsonic index
23 cases of carcinomata	0·96
4 „ sarcomata	0·92
5 „ healthy medical men	0·85

With *M. neoformans* :—

25 cases of carcinomata	0·75
4 „ sarcomata	0·85
5 „ healthy medical men	0·86

The serum from a case of ulcerating carcinoma of the colon (case 5) was employed for this purpose (serum 141). Serum 141 + equal parts of the *M. neoformans* was digested for one hour at 37° C. and then centrifugalised; in this way a deposit and a supernatant liquid (serum A) were obtained. The opsonic content of the patient's serum for the *M. neoformans* and *Staph. albus* was as follows :—

	Cocci contained in 50 phagocytes
Serum 141 + <i>M. neoformans</i> (epithelioma) + leucocytes	155
„ 141 + <i>Staph. albus</i> + leucocytes	197
„ 141 + <i>M. neoformans</i> (sarcoma) + leucocytes	145
„ A + <i>M. neoformans</i> (epithelioma) + leucocytes	21
„ A + <i>Staph. albus</i> + leucocytes	29
„ A + <i>M. neoformans</i> (sarcoma) + leucocytes	12

These experiments serve to show that there is no specific opsonic substance in immune serum for either the *M. neoformans* or for the *Staph. albus*. This is what we were led to expect from other experiments made during this investigation.

The average *Staph. albus* opsonic index in cases of carcinoma and sarcoma appears, from our investigations, to be higher than in healthy men, although in all instances the average is below 1, but within the normal limits. On the other hand, the *M. neoformans* opsonic index in cases of carcinoma is much lower than in the cases of the *Staph. albus* index. The one case among the “healthy” medical men who had a low *M. neoformans* opsonic index (0·4), was afterwards found to be suffering from furunculosis on the back. This fact is of great interest, considering that the *Staph. albus* opsonic index was within the normal limit, and also in view of the important part which the organism has been considered to play in malignant disease. In the large majority of cases in which the opsonic index was determined, ulceration of the neoplasm was found to have occurred.



*Inoculation Experiments.*

We have made very few inoculation experiments, as this branch of the subject has been so fully dealt with by Paine and Morgan.

*Experiment 1.* 1 c.c. of a 24 hours' agar growth (emulsified in sterile normal saline) was injected into the peritoneal cavity of a guinea-pig. The animal was ill for a few days, but complete recovery followed. A second inoculation was made three weeks later, a similar result ensued.

The animal was killed at the end of one month after the date of the first inoculation. At the post-mortem examination, the animal's body was quite healthy. There were no nodules on the peritoneum or elsewhere. A bacteriological examination was made of the heart-blood and peritoneal fluid. All the cultures were found to be sterile. Film preparations made with the peritoneal fluid showed a few cells which were almost entirely macrophages.

*Experiment 2.* A guinea-pig was inoculated in a similar manner as in the above experiment. The animal was killed three weeks later. At the post-mortem examination nothing abnormal was detected.

*Experiment 3.* Three mice received intraperitoneal injections of 2 c.c. of the *M. neoformans*. Three weeks later, they received a second inoculation and only showed a slight illness for the first twenty-four hours. All three animals were killed at the end of seven weeks from the date of the first inoculation. At the post-mortem examination, they were found to be quite healthy.

Paine and Morgan inoculated 200 animals (110 mice and 90 rats) intraperitoneally. They failed to find any evidence of simple or malignant tumours as the result of these inoculations, although many of the animals were kept alive for three months and some for six months.

Our inoculation experiments are so few that the results cannot be compared with those obtained by either Doyen himself or by Paine and Morgan, but they are similar to those of the latter observers.

*Conclusions.*

1. Our results appear to bear out the conclusion that the *Micrococcus neoformans* is an organism which is only occasionally met with in simple inflammatory affections. Although not identical with the so-called *Staphylococcus pyogenes albus* or the *Staphylococcus epidermidis albus* (Welch), it is closely related to these organisms.

2. The cultural properties of the organism obtained from various sources are identical. (This applies to three cases.)

3. The serum of patients suffering from malignant disease, although the neoplasm may have ulcerated, does not develop any very marked agglutinative property for the *M. neoformans*, in fact it is less than that which is formed for the *Staph. albus*.

4. There are no specific opsonins for *M. neoformans* and the *Staph. albus*. These cocci appear to have about an equal power for removing the opsonic substance in immune sera which is present for them.

5. *M. neoformans* is an organism of very low pathogenicity.



THE RESULTS OF A CHEMICAL, MICROSCOPICAL AND  
BACTERIOLOGICAL EXAMINATION OF SAMPLES OF  
LONDON MILKS.

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IN view of the importance of a pure milk supply, we considered that it might be of interest to examine chemically, microscopically, and bacteriologically, a number of specimens of milk coming into the Metropolis for which purpose we decided to select samples from the various counties, the milk of which is consigned to London. We found that milk so consigned comes from about twenty-six counties extending from Derby in the North, to Hampshire and Devonshire in the South and South-West, and from Hereford in the West, to Norfolk in the East.

*Description of manner of obtaining samples.*

The samples were collected on arrival in the early morning at the various railway termini, viz. Euston, St Pancras, Liverpool St., Waterloo, Marylebone, etc., by the inspectors and samplers of several well known Dairy Companies. Two sterilised eight-ounce bottles with new, good sound corks, were used for each specimen, the contents of one bottle being used for the chemical and those of the other for the bacteriological examination, thus obviating any possibility of contamination. The milk contained in a churn was thoroughly roused with a clean plunger; the bottles were immersed, recorked, labelled and marked with the time of collection, date, the particular county in which

it had been produced and the company's name. They were then transmitted without delay to the laboratory, and the examination at once commenced.

The *chemical examination* consisted in determining the specific gravity, the percentage of fat, the non-fatty solids and the total solids, the acidity, and a search for preservatives, viz. formalin, boric acid, and borates.

*Microscopically*; acid-fast organisms, streptococci, pus-cells, and *débris* were looked for.

*Culturally*; a search was made for the *Bacillus enteritidis sporogenes* (with subsequent inoculation of typical cultures into guinea-pigs), for the *Bacillus coli* in definite quantities of the sample, and for the *Bacillus diphtheriae* and diphtheroid organisms in the sediment which was finally inoculated into guinea-pigs to test for tubercle and other pathogenic organisms.

#### *Brief description of Methods employed.*

1. Determination of specific gravity; the Westphal Balance.
2. Estimation of the fat; Gerber's method.
3. Total solids; calculated by the formula,  $T = .25G + 1.2F + .14$ , and the non-fatty solids by difference.
4. The test for formalin employed was the ordinary sulphuric acid reaction, any doubtful ones being controlled by Schiff's method.
5. For boric acid, the turmeric test was used, confirmed by the phenolphthalein-glycerine method.

6. The acidity was estimated by running into 50 c.c. of the sample  $\frac{N}{10}$  sodium hydrate, phenol-phthalein being used as the indicator, each 0.5 c.c. representing 1 degree of acidity: this multiplied by .009 gives the percentage estimated as lactic acid (Richmond).

For the *microscopical* examination, 100 c.c. of the sample were centrifugalised for 15 minutes at about 1000 revolutions per minute, smears were then made from the deposit, the fat was removed by treating the films with a mixture of equal parts of ether and alcohol. They were then dried and fixed by heat, and examined for acid-fast organisms by the Ziehl-Neelsen method.

For streptococci, the films were stained by Gram's method, (Nicolle's modification with carbol-thionin blue) and for pus-cells and leucocytes with Löffler's methylene blue, and *débris* was looked for in wet specimens.

For the *Bacillus enteritidis sporogenes*, quantities of 1 c.c., 10 c.c. and 20 c.c. were examined. The 1 c.c. was added to a tube of sterile milk, the 10 c.c. and 20 c.c. were placed in sterile test tubes, and all the tubes were heated to 80° C. for 15 mins., and incubated anaerobically at 37° C. for 48 hours, the typical cultures being subsequently inoculated subcutaneously into the abdominal region of a guinea-pig.

For the *Bacillus coli*, quantities of 1 c.c., 0·1 c.c., 0·01 c.c., and 0·001 c.c. were added to tubes of litmus lactose bile salt peptone water and incubated at 42° C. for 48 hours. From the tube containing the least amount which gave acid and gas a loopful was inoculated into 10 c.c. of sterile water, and from this a loopful was smeared upon a slope gelatin tube. This always gave isolated colonies, and from a characteristic colony inoculations were made into broth, peptone water, 1 % lactose peptone water, 1 % glucose peptone water, 1 % mannitol peptone water, 1 % cane sugar peptone water, and 1 % dulcitol peptone water. The gelatin tubes were kept during the whole of the experiments and not one liquefied.

For the estimation of the number of organisms present a dilution of 1 in 10,000 was made in sterile water, and 1 c.c., 0·5 c.c., 0·1 c.c. of this dilution was dropped into a sterile Petri dish, and nutrient gelatin of reaction +1·5 (Eyre's scale) added, and incubated at 20° C. for 48 hours.

For *Bacillus diphtheriae* and diphtheroid organisms, loopfuls of the sediment of the centrifugalised milk were inoculated upon blood serum, and examined after incubation in films stained with Löffler's methylene blue, and by Neisser's method.

For tubercle and other pathogenic organisms, 50 c.c. of the milk were centrifugalised for 20 minutes at 1000 revolutions per min., and the upper portion poured off, leaving only 3 c.c., which was thoroughly mixed, and of it 1·5 c.c. was inoculated subcutaneously into one guinea-pig, and the remainder intraperitoneally into a second guinea-pig. Owing to the premature death of some of the animals, duplicate samples from the same source were procured, and the experiment repeated.

The following table (Table 1) sets forth the names of the counties from which the milk was taken, the date of the collection, the specific gravity, and the percentages of the fats, of the non-fatty solids and of the total solids; also the acidity after 24 hours, and the presence or absence of formalin or boric acid, which last are expressed by positive or negative signs.

From Table 1 it will be seen that six of the twenty-six samples (23 per cent.) failed to come up to the Departmental Committee's standards, three in respect of fats, and three in respect of the non-fatty solids. Notwithstanding, the average of all the samples is very good. Preservatives in the form of formalin, boric acid and borates were not detected in any sample.

As regards the acidity, Newman and Swithinbank have suggested as a standard that no milk should have an acidity corresponding to more than 22 c.c. of  $\frac{N}{10}$  NaOH for 50 c.c. of the milk at time of sale. It will be seen that seven of the samples exceeded this (26·9 per cent.). The remainder (73·1 per cent.) are well below it. Our test, however, was a rigorous one, the milk being kept for 24 hours before the acidity was tested, whereas actually under normal conditions, all the milk should have been disposed of within 24 hours.

TABLE I.

County	Date	Sp. gr.	Fat	Solids not fat	Total solids	Acidity after 24 hours	Degrees of acidity	Formalin	Boric acid and borates	Remarks
Leicester	1. iii. 06	1032.6	2.95	8.89	11.84	.198	22.0	—	—	1.7% deficient in fat.
Northampton	"	1031.6	3.58	8.76	12.32	.172	19.2	—	—	
Derby	2. iii. 06	1032.6	3.08	8.91	11.99	.208	23.2	—	—	
Stafford	"	1032.5	3.00	8.88	11.88	.189	21.0	—	—	
Essex	5. iii. 06	1034.0	5.30	9.80	15.10	.201	22.4	—	—	
Somerset	8. iii. 06	1031.4	3.62	8.72	12.34	.171	19.0	—	—	
Gloucester	"	1030.9	3.40	8.54	11.94	.138	15.4	—	—	
Oxford	9. iii. 06	1031.4	2.70	8.67	11.37	.163	18.2	—	—	10% deficient in fat.
Buckingham	"	1030.0	3.50	8.35	11.85	.218	24.3	—	—	1.8% water.
Suffolk	10. iii. 06	1033.7	4.10	9.39	13.49	.170	18.9	—	—	
Hampshire	"	1030.9	4.10	8.69	12.79	.167	18.6	—	—	
Sussex	15. iii. 06	1032.0	3.84	8.90	12.74	.163	18.2	—	—	
Berkshire	"	1033.1	3.25	9.07	12.32	.158	17.6	—	—	
Bedford	16. iii. 06	1033.0	3.47	9.09	12.56	.178	19.8	—	—	
Warwick	"	1033.4	3.85	9.26	13.11	.203	22.6	—	—	
Kent	25. iv. 06	1028.8	4.95	8.34	13.29	.167	18.6	—	—	1.9% water.
Wiltshire	"	1032.0	3.56	8.85	12.41	.169	18.8	—	—	
Norfolk	26. iv. 06	1034.5	4.22	9.61	13.83	.172	19.2	—	—	
Cambridge	"	1032.1	3.67	8.91	12.58	.178	19.8	—	—	
Middlesex	30. iv. 06	1031.4	2.80	8.55	11.35	.180	20.0	—	—	6.7% deficient in fat.
Survey	"	1032.2	3.98	8.98	12.96	.225	25.0	—	—	
Dorset	4. v. 06	1029.8	3.54	8.32	11.86	.151	16.8	—	—	2.2% water.
Cheshire	"	1030.9	3.22	8.50	11.72	.169	18.8	—	—	
Devon	11. v. 06	1032.6	4.40	8.97	13.37	.187	20.8	—	—	
Hereford	"	1032.0	3.80	9.62	13.42	.219	24.4	—	—	
Rutland	"	1032.8	3.80	8.98	12.78	.206	22.9	—	—	
Average		1032.0	3.68	8.90	12.56	.181	20.2			

As the temperature is of considerable importance as regards the rate of acid production the following table (Table 2) shows the maximum and minimum temperatures taken at the Lancet Office and recorded in the *Lancet*, Vol. I. 1906, for the day preceding, the day of, and the day following the collection and examination of every sample. It will be seen that the temperatures on the whole were moderate, the maximum exceeding 60° F. on only five occasions.

TABLE II.

Samples	Date	Shade Maximum	Minimum
	28. ii. 06	46	35
I & II.	1. iii. 06	49	37
III & IV.	2. iii. 06	52	46
	3. iii. 06	49	35
	4. iii. 06	58	37
V.	5. iii. 06	53	36
	6. iii. 06	65	43
	7. iii. 06	67	48
VI & VII.	8. iii. 06	51	47
VIII & IX.	9. iii. 06	50	42
X & XI.	10. iii. 06	47	39
	11. iii. 06	54	41
	14. iii. 06	51	30
XII & XIII.	15. iii. 06	55	35
XIV & XV.	16. iii. 06	57	50
	17. iii. 06	66	47
	24. iv. 06	48	36
XVI & XVII.	25. iv. 06	51	39
XVIII & XIX.	26. iv. 06	49	36
	27. iv. 06	56	35
	29. iv. 06	52	35
XX & XXI.	30. iv. 06	49	37
	1. v. 06	53	37
	3. v. 06	59	47
XXII & XXIII.	4. v. 06	60	50
	5. v. 06	63	45
	10. v. 06	49	46
XXIV, XXV, & XXVI.	11. v. 06	58	47
	12. v. 06	71	50

Table 3 gives the number of micro-organisms per cubic centimetre, the presence or absence of acid-fast organisms, streptococci, leucocytes and pus-cells, and the results of growth on blood serum.



TABLE III.

County	Organisms per c.c.	Acid-fast organisms	Strepto- cocci	Leucocytes and pus-cells	Diphtheria organisms on blood serum cultures	Débris
Leicester	240,000	—	—	Very few	—	1
Northampton	230,000	—	Few	Few	—	2
Derby	1,400,000	—	—	„	+ (1)	3
Stafford	5,000,000	—	—	„	— (2)	4
Essex	60,000	—	—	Very few	— (3)	5
Somerset	1,480,000	—	Many	Few	+ (4)	6
Gloucester	56,000	—	—	„	—	7
Oxford	143,000	—	—	„	+ (5)	8
Buckingham	7,810,000	—	—	Very few	—	9
Suffolk	96,000	—	—	Few	—	10
Hampshire	50,000	—	—	„	—	11
Sussex	184,000	—	—	„	—	12
Berkshire	50,000	—	—	Very few	—	13
Bedford	100,000	—	—	Few	—	14
Warwick	20,000	—	—	Very few	—	15
Kent	8,390,000	—	—	Few	+ (6)	16
Wiltshire	340,000	—	—	„	—	17
Norfolk	250,000	—	—	„	—	18
Cambridge	120,000	—	—	„	— (7)	19
Middlesex	1,830,000	—	—	„	— (8)	20
Surrey	1,443,000	+	Very many	Many	— (9)	21
Dorset	1,560,000	—	Many	Few	—	22
Cheshire	20,000	—	—	„	—	23
Devon	880,000	—	—	„	—	24
Hereford	1,510,000	—	—	„	—	25
Rutland	2,800,000	—	—	„	—	26

References to "blood serum cultures":

- (1) Somewhat similar to the Klebs-Löffler bacillus, but much too large.
- (2) Many yeasts or torulae.
- (3) A few yeast-cells.
- (4) Somewhat like Klebs-Löffler bacillus; not identical.
- (5) " " " " " " " " " " " "
- (6) Much like Klebs-Löffler bacillus in parallel grouping, polar staining by Löffler's methylene blue, and by Neisser's method; failed to isolate the organism.
- (7) Many large capsulated diplococci in deposit.
- (8) On blood serum tube many streptococci and leptothrix forms. The indol reaction was intense in a broth culture.
- (9) Acid-fast organisms and streptococci present.

References to "débris":

- (1) Yeast-cells, cotton fibre, vegetable matter.
- (2) " " " " " " " " " " " "
- (3) Vegetable matter, hairs. " " (4) Epithelial cells, vegetable matter.
- (5) Wool, vegetable matter. (6) Grit and hairs.
- (7) Vegetable matter, cotton fibre. (8) Hairs, epithelial cells.
- (9) Cotton fibre. (10) Hair, vegetable matter. (11) Antennae, ? of a fly.
- (12) Antennae, ? of a fly. (13) Wool, grit. (14) Grit, cotton fibre.
- (15) Straw. (16) Vegetable matter. (17) Vegetable matter.
- (18) Grit. (19) Vegetable matter. (20) Hair.
- (21) Cotton fibre. (22) Epithelial cells. (23) Yeast-cells.
- (24) Grit. (25) Cotton fibre. (26) Cotton fibre.

The above table shows great variation in the total number of organisms per c.c. from 20,000 to 8,390,000; streptococci and leucocytes and pus-cells were scanty with one or two exceptions, and acid-fast bacilli only detected once.

Table 4 shows the production of acid and gas (A & G) in their respective quantities, in the lactose bile salt tubes. (When the sign is bracketed it is to indicate that the change was a slight one.)

TABLE IV.

	Quantity:—1 c.c.	0·1 c.c.	0·01 c.c.	0·001 c.c.
Leicester	A & G	A & G	—	—
Northampton	A & G	A & G	—	—
Derby	A & G	A & G	A & G	A & G
Stafford	A & G	A & G	(A & G)	—
Essex	A & G	A & G	A & G	—
Somerset	A & G	A & G	A & G	—
Gloucester	A & G	A & G	A & G	—
Oxford	A & G	A & G	—	—
Buckingham	A & G	(A & G)	(A & G)	(A & G)
Suffolk	A & G	A & G	A & G	—
Hampshire	A & G	A & G	A & G	(A)
Sussex	A & G	A & G	A & G	—
Berkshire	A & G	A & (G)	—	—
Bedford	A & G	A & G	A & G	—
Warwick	A & G	A & G	—	—
Kent	A & G	A & G	A & G	A & G
Wiltshire	A & G	A	A	A
Norfolk	A & G	A	(A)	—
Cambridge	A & G	A	—	—
Middlesex	A & G	A & G	—	—
Surrey	A & G	A & G	(A)	—
Dorset	A & G	A & G	A & G	A & G
Cheshire	A & G	A & G	A & G	(A)
Devonshire	A & G	A & G	A	—
Hereford	A & G	A & (G)	A & G	A & G
Rutland	A & G	A & (G)	A & (G)	A & (G)

All the twenty-six samples (100 per cent.) therefore contained lactose fermenting (acid and gas) organisms in 1 c.c., 23 (88 per cent.) in 0·1 c.c., 15 (57 per cent.) in 0·01 c.c., and 6 (23 per cent.) in 0·001 c.c.



Table 5 gives the attributes, *i.e.* indol in peptone water, acid and gas in :

	1% lactose	peptone	water
"	"	glucose	"
"	"	mannitol	"
"	"	cane-sugar	"
"	"	dulcitol	"

of the organisms isolated from the lactose bile salt tubes.

TABLE V.

County	Peptone Water	In peptone water					Organisms probably isolated, and from what amount
		Lactose	Glucose	Mannite	Cane sugar	Dulcite	
Leicester	in	A & G	A & G	A & G	—	A & G	<i>B. coli</i> .1 c.c.
Northampton	(in)	A & G	A & G	A & G	—	A & G	" .1 c.c.
Derby	—	A & G	A & G	A & G	—	—	<i>B. ac. lactici</i> .
Stafford	—	A & G	A & G	A & G	A & G	A & G	<i>B. coli</i> .1 c.c.
Essex	in	A & G	A & G	A & G	A & G	—	<i>B. lact. aerog.</i>
Somerset	—	A & G	A & G	A & G	(A & G)	(A) & G	<i>B. coli</i> .01 c.c.
Gloucester	—	A & G	A & G	A & G	—	(A) & G	" .01 c.c.
Oxford	—	A & G	A & G	A & G	—	A & G	" .1 c.c.
Buckingham	in	A & G	A & G	A & G	A	A & G	" .1 c.c.
Suffolk	—	A & G	A & G	A & G	—	—	<i>B. ac. lactici</i> .
Hampshire	in	A & G	A & G	A & G	—	—	" "
Sussex	in	A & (G)	A & G	A & G	A & G	—	<i>B. lact. aerog.</i>
Berkshire	in	A	A	A & G	A & G	A & G	?
Bedford	(in)	A & G	A & G	A & G	—	—	<i>B. ac. lactici</i> .
Warwick	in	(A)	A	A	(A)	A & G	?
Kent	in	A	A	A & G	A & G	A & G	?
Wiltshire	—	A & G	A & G	A & G	—	A & G	<i>B. coli</i> 1 c.c.
Norfolk	(in)	(A)	A	—	(A)	—	?
Cambridge	—	A & G	A & G	A & G	—	A & G	<i>B. coli</i> 1 c.c.
Middlesex	(in)	—	A	—	A	—	?
Surrey	—	A & G	A & G	A & G	—	A & (G)	<i>B. coli</i> .1 c.c.
Dorset	in	A & G	A & (G)	A & G	A & G	—	<i>B. lact. aerog.</i>
Cheshire	in	A & G	A & G	A & G	(A)	A & G	<i>B. coli</i> .01 c.c.
Devonshire	—	A & G	A & G	A & G	—	—	<i>B. ac. lactici</i> .
Hereford	—	A & G	A & G	A	(A)	A & G	<i>B. coli</i> .001 c.c.
Rutland	—	A & G	A & G	A & G	A & G	—	<i>B. lact. aerog.</i>

The diagnosis of the organisms from the fermentation tests in Table 4 is based on MacConkey's researches<sup>1</sup>. According to him the *B. coli* ferments dulcitol and mannitol, it may or may not ferment cane-sugar; the *B. lactis aerogenes* ferments mannitol and cane-sugar, but not dulcitol; the *B. acidi lactici* ferments mannitol but not dulcitol nor

<sup>1</sup> MacConkey (1905.) *Journal of Hygiene*, Vol. v. p. 333.

cane-sugar. All the organisms were bacilli, not staining by Gram's method (Nicolle's modification with carbol-thionin blue). No gelatin culture showed liquefaction. The *B. coli* was therefore definitely found in twelve of the samples (46 per cent.) and the *B. lactis aerogenes* in 4 (15·4 per cent.).

*B. coli* was found three times (11·5 per cent.) in not less than 1 c.c., four times (15·4 per cent.) in 0·1 c.c., twice (8 per cent.) in 0·01 c.c., and three times (11·5 per cent.) in 0·001 c.c.

Table 6 shows the results of the *B. enteritidis sporogenes* test. The contents of the tubes containing the smallest amount showing the characteristic change were inoculated into guinea-pigs.

TABLE VI.

County	1 c.c.	10 c.c.	20 c.c.	Remarks
Leicester	—	+	+	Animal negative. (10 c.c.)
Northampton	—	+	+	„ typical. (10 c.c.)
Derby	+	+	+	„ negative. (1 c.c.)
Stafford	—	+	+	„ „ (10 c.c.)
Essex	—	+	+	„ „ (10 c.c.)
Somerset	—	+	+	„ typical. (10 c.c.)
Gloucester	—	—	+	„ „ (20 c.c.)
Oxford	—	—	—	Cultures not typical.
Buckingham	—	—	+	Animal typical. (20 c.c.)
Suffolk	—	—	—	Cultures not typical.
Hampshire	—	+	+	Animal typical. (10 c.c.)
Sussex	—	—	+	„ negative. (20 c.c.)
Berkshire	+	+	+	„ typical. (1 c.c.)
Bedford	—	—	—	Cultures not typical.
Warwick	—	—	+	Animal typical. (20 c.c.)
Kent	—	+	+	„ „ (10 c.c.)
Wiltshire	—	—	—	Cultures not typical.
Norfolk	—	+	+	Animal typical. (10 c.c.)
Cambridge	+	+	+	„ negative. (1 c.c.)
Middlesex	—	+	+	„ „ (10 c.c.)
Surrey	—	+	+	„ typical. (10 c.c.)
Dorset	—	+	+	„ „ (10 c.c.)
Cheshire	—	—	+	Tube broken, no inoculation.
Devon	—	—	—	Cultures not typical.
Hereford	—	+	+	Animal typical. (10 c.c.)
Rutland	—	+	+	„ negative (10 c.c.)

From Table 6 it will be seen that in one instance the tube was broken and no inoculation could therefore be made. Of the 26 samples 21 (80·8 per cent.) showed the enteritidis change in 20 c.c. or less. Excluding the broken tube, of the 20 samples showing the enteritidis change and tested by inoculation 12 (60 per cent.) gave a positive

result on the animal. Therefore, one-fifth of the number showing the enteritidis change culturally proved not to be infected with the enteritidis by animal inoculation.

As regards tuberculosis, only one sample, Surrey, out of the 26 (about 4 per cent.) gave definite evidence of tubercle bacilli. The inoculated guinea-pig died with typical tubercle and acid-fast bacilli were found in the milk, which contained an excess of streptococci and leucocytes and pus-cells. The cow was subsequently identified on the farm, and the further use of its milk stopped.

Although we have not alluded in this paper to the work of others on the bacterial content of milk, etc., we are by no means unmindful of the numerous valuable contributions which have been made on the subject. We would refer particularly to Houston's report<sup>1</sup> which contains a summary of previous work.

#### *Conclusions.*

As regards the general results of the examination it may be said :

1. There is no correlation between poor milk and its content of total bacteria, *B. coli* or *B. enteritidis sporogenes*.

2. There is no correlation between the content of *B. coli* and of *B. enteritidis sporogenes*.

3. The total number of organisms was below 2,000,000 per c.c. in 22 out of the 26 samples (85 per cent.) and below 1,000,000 in 16 of the samples (61·5 per cent.).

4. *B. coli* was found in 46 per cent. of the samples, in a quantity of milk not exceeding 1 c.c.

5. *B. enteritidis sporogenes* was found in 60 per cent. in a quantity of milk not exceeding 20 c.c.

6. Preservatives in the form of formalin, or boric acid, or borates, were not detected in any sample.

7. The acidity on the whole is well below Newman's standard.

8. *B. tuberculosis* was not so frequent as might have been expected from the results of other investigators.

In conclusion we desire to express our best thanks to the various companies, etc., who supplied us with samples, and particularly to the company supplying the Surrey milk. The latter, when we found acid-fast bacilli, etc., in the sample, gave us every facility for examining the herd and we were thus able to identify the infected animal.

<sup>1</sup> Houston, A. C. "The Bacteriological Examination of Milk." *Report No. 933 to the London County Council.*

## NOTE ON THE OCCURRENCE OF DIPHTHERIA BACILLI IN MILK.

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THOUGH milk has often been considered the medium by means of which outbreaks of diphtheria have been propagated, the diphtheria bacillus has only on rare occasions been isolated from the milk in a virulent form.

Bowhill (1899), Eyre (1899), Klein (1901), and Dean and Todd (1901) are at present the only authors who instance cases and there seems to be no reference to similar cases apart from English literature. In June of this year there was received at this Institute for bacteriological examination a sample of milk, supposed to be associated with an outbreak of diphtheria, from which the diphtheria bacillus was isolated in a virulent form. The outbreak consisted of two cases of diphtheria, with an interval of about six months between them, which occurred in a private house with a private dairy attached. After milking, the milk was conveyed to the milk-house, which is situated in the basement of the house, poured into a sterilised bottle and forwarded to the Institute.

Four guinea-pigs were inoculated subcutaneously with the centrifuged deposit of the sample, two being inoculated on the 12th June 1906 and two on the 13th. On the morning of the 16th these four guinea-pigs were all dead. The post-mortem appearances presented by these guinea-pigs were characteristic of diphtheria. Subcultures were made on inspissated blood-serum from the local lesions at the site of inoculation and in guinea-pigs Nos. I and III a bacillus morphologically resembling the Klebs-Löffler Bacillus of Diphtheria was found and isolated on agar plates.

The virulence and specificity of these two strains, Nos. I and III, were tested as follows:



In all the guinea-pigs which died from inoculation the post-mortem appearances were typical of diphtheria infection and in guinea-pigs Nos. 2, 3, 7 and 8 the bacillus of diphtheria was again isolated from the tissues.

This seems conclusive proof that the diphtheria bacilli were present in the milk in a virulent form. As to the source of the bacilli and their mode of entrance into the milk one is unable in the present case to come to a definite conclusion. Dean and Todd, and more recently Ashby, have described outbreaks of diphtheria, in the latter instance consisting of 75 cases. In both outbreaks ulceration of the teats of the cows supplying the milk was associated with the presence of virulent bacilli on the lesions, so that it seemed important to enquire into the condition of the udders and teats of the cows supplying the milk in this instance. I had the privilege, along with a veterinary surgeon, of inspecting the dairy and all the animals connected with it.

In all the cows the teats and udders appeared perfectly healthy and only in one cow did we find anything resembling what may have been the scar of a previous ulcer. Swabs taken from the throats of the milkman and dairymaid afforded negative results upon examination.

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## ON THE SUPERSENSITISATION OF PERSONS SUFFERING FROM DIPHTHERIA BY REPEATED INJECTIONS OF HORSE SERUM.

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WHEN the blood serum of one animal is injected into an animal of a different species the injected animal in many instances appears to take no hurt. If however after a certain interval the experiment is repeated, it has been noted that the injected animal may speedily show evidence of physical disturbance. Its breathing becomes rapid: its heart-beat grows feeble: its limbs move spasmodically and general convulsions may ensue. These phenomena have been taken to indicate that the injected animal has been *supersensitized*—or rendered abnormally sensitive—to the serum employed. They may attain such gravity that the animal dies. It has been suggested that the same untoward issue is possible in the case of man, and that the death of patients under treatment for diphtheria is to be apprehended in certain circumstances as the result of repeated injections of antidiphtherial serum, for the reason that the serum in question is derived not from the human subject but from the horse. A suggestion which assails the prestige of the antitoxin treatment of diphtheria cannot be viewed with indifference: it must either be sustained or rejected.

The narratives of the cases of persons who have received such repeated injections are criteria for the discussion of the question whether the mischance suggested is an imminent danger or is merely a speculative possibility. It thus seemed appropriate to extract from a continuous series of reports such information as was pertinent in the matter. It is the purpose of this paper to record the effects of repeated injections of horse serum in persons associated with the City of Glasgow Fever and Small-pox Hospitals at Belvidere.

It may be permitted by way of initial survey to recall the work of certain observers, to refer in the first place to experiments on supersensitisation, or production of excessive susceptibility, in animals by bacterial products and by extraneous sera, and in the second place to investigations of the corresponding phenomena in the human subject.

*Supersensitisation of animals to bacterial products.*

Brieger<sup>(6)</sup> (according to Otto, p. 9) relates the case of a goat which died of typical tetanus, while highly immunized against the toxin of that disease.

Von Behring and Kitashima (1901) record the death of a horse in the course of immunization against the diphtheria toxin, despite the high antitoxic content of its blood.

Rist (1903) narrates the effects produced in guinea-pigs by the repeated injection of .01 to .05 cg. of dried diphtheria bacilli. In these experiments the first dose caused illness and recovery; the second dose caused illness with a slower recovery; the third dose was followed by death.

It was demonstrated by Kretz<sup>(10)</sup> that an estimated mixture of toxin with anti-toxin which was neutral when injected into normal animals induced a reaction in animals which had been actively immunized to the toxin.

The repeated tuberculinization of tuberculous cattle was stated by Nocard<sup>(12)</sup> to effect a genuine tolerance. Vallée<sup>(13)</sup> was led to an opposite view by experiments which are traversed by Arloing, but which seem to indicate that a second or third injection of tuberculin into a tuberculous ox produces a reaction which is earlier in its appearance and more rapid in its course than the reaction which follows a first injection.

It emerges from the instances related that an animal under certain circumstances may be supersensitized, or rendered more sensitive than normal, to the action of bacteria and their products, and that the condition so produced may be displayed either in a fatal issue, or in the occurrence of a phenomenon which would otherwise fail to appear, or in the earlier onset of the reaction induced.

*Supersensitisation of animals to extraneous sera.*

Arthus (1903) conducted a series of experiments in which the repeated injection of rabbits with normal horse serum produced a specific supersensitisation. One rabbit died of the intravenous injection of 2 c.c. of horse serum. Under this reference Arthus suggests the possibility of danger to man by similar treatment.

A further inquiry by Arthus with Breton (1903) demonstrated the occurrence of severe cutaneous lesions in rabbits supersensitized to horse serum, as a result of repeated injections of the serum.

Wolf (cited by Otto, p. 11) has shown that the repeated injection of extraneous serum does not produce tolerance: on the contrary the animals progressively

deteriorate. He regards the condition as supersensitisation to a specific serum and not as an impairment of defence against noxious substances in general.

The researches of Battelli (1905) are concerned with blood corpuscles. They may however be noted here. These researches indicate that the extractives of dogs' or guinea-pigs' blood corpuscles which are not toxic to normal rabbits are capable of causing the death of rabbits which have been immunized against dogs' or guinea-pigs' blood corpuscles.

It appears by these records of the repeated injection into animals of the sera and blood extractives of animals of a different species that a condition of supersensitisation results in the injected animals, which is similar in character to that which follows the repeated injection of bacterial products.

Reference may be made in more detail to the recent inquiry of Otto<sup>(13)</sup> which is concerned with diphtheria toxin and with horse serum. Six series of experiments on guinea-pigs constitute this inquiry. The deaths narrated occurred as a rule within an hour or thereby of the second injection.

*Series I*, establishing the phenomenon, which is associated by Otto with the name of Theobald Smith. 22 guinea-pigs. *First injection*, horse serum '002 to '0025 c.c. with diphtheria toxin,  $L$ +dose, that is to say, the amount of diphtheria toxin which when mixed with one unit of standard antitoxin causes the death of a 250 gramme guinea-pig on the 4th or 5th day. *Second injection*, normal horse serum 6 c.c. *Interval*,  $4\frac{1}{2}$  to 12 weeks. *Result*, 50 % of deaths.

*Series II*, also establishing the phenomenon. 14 guinea-pigs. *First injection*, horse serum '235 to 6'4 c.c. with toxin  $\frac{2}{3}$  to 19 times  $L$ +dose. *Second injection*, normal horse serum 6 c.c. *Interval*, 6 to 14 weeks. *Result*, all reacted, 6 died.

*Series III*, excluding sera other than horse serum. 16 guinea-pigs. *First injection*, horse serum with toxin. *Second injection*, rabbit serum 6 c.c. into 2; goat serum 10 c.c. into 11; ox serum 30 c.c. into 3. *Interval*, 5 to 10 weeks. *Result*, none reacted.

*Series IV*, excluding toxin. 34 guinea-pigs. *First injection*, toxin  $\frac{1}{5}$  to  $\frac{3}{5}$   $L$ +dose. *Second injection*, normal horse serum 6 c.c. *Interval*, 4 to 11 weeks. *Result*, 32 gave no reaction, 2 died.

*Series V*, excluding toxone. 11 guinea-pigs. *First injection*, toxone. *Second injection*, normal horse serum. *Interval*, 6 to 10 weeks. *Result*, none reacted.

*Series VI*, isolating the specific agent. 32 guinea-pigs. *First injection*, normal or antidiphtheritic horse serum '0025 to 10 c.c. *Second injection*, normal horse serum 6 to 7 c.c. *Interval*,  $4\frac{1}{2}$  to 14 weeks. *Result*, guinea-pigs which had a large first dose showed no reaction. Guinea-pigs which had a small first dose reacted. None of series VI died.

There are clear deductions from Otto's experiments. Supersensitisation is induced by horse serum. The reaction is specific for horse serum. Small first injections are more effective than large. Neither toxine nor toxone plays an essential part, though toxine appears to intensify. An interval of two weeks to two or three months must elapse between the injections. Otto determines further that the reaction is not due to precipitins for precipitins were not found.

Rosenau and Anderson (1906, p. 179) furnish details to show that the guinea-pig is more susceptible to two injections of the same serum than to two injections of sera derived from different species of animals. The phenomenon in their phrase is quantitatively and not absolutely specific. In other respects Rosenau and Anderson sustain Otto's deductions. They further suggest that supersensitisation is induced in guinea-pigs by feeding with horse serum or with horse flesh, and that it is transmitted by the mother to her young. Anderson (1906, p. 259) in another paper records experimental evidence of this transmission.

It appears from the experiments of Otto and of Rosenau and Anderson that the repeated injection of guinea-pigs with horse serum induces a specific condition which is of the same order as the supersensitisation effected by other extraneous sera and by bacterial products.

*Corresponding phenomena in the human subject.*

The repeated injection of horse serum for diphtheria of the human subject offers conditions analogous to those detailed for guinea-pigs. In the case of repeated injection of the human subject a fatal result is not reported by authors. Otto (p. 18) however, and von Pirquet and Schick (1905, p. 98) each record a case which came near to death, and Rolleston (1905, p. 664) refers to grave symptoms which may ensue within a few hours of injection in cases of relapse or of a second attack of diphtheria. Nevertheless while extreme severity is uncommon, the course of events which follows the administration to man of two suitable injections of horse serum differs in a more or less constant manner from the sequence after one administration.

The phenomena are minutely studied by von Pirquet and Schick (p. 98) in their work on the serum disease. Cases which react to two injections of serum are classed by these observers in three divisions: first, cases which show an immediate reaction only: second, cases which show an immediate reaction and an accelerated reaction: third, cases which show an accelerated reaction only. The immediate reaction is marked specifically by a local oedema of varying degree. The reaction is also attended by erythema, urticaria, constitutional disturbance and the like. It is apparent within 24 hours of injection. The accelerated reaction is described as a train of symptoms which differ from the results of a single injection in their earlier onset, briefer duration and frequently severer course.

Of 61 cases which are presented by von Pirquet and Schick in a tabular form as having received two injections of serum, 60 are suitable for classification in accordance with their predominant reaction.

*First Division.* Immediate reaction. 30 cases. Interval between injections of serum 12 to 50 days.

*Second Division.* Immediate reaction with accelerated reaction. 11 cases. Interval between injections of serum 2 to 6 months.

*Third Division.* Accelerated reaction. 19 cases. Interval between injections of serum 7 months to  $7\frac{1}{2}$  years.



The *First Division* shows the immediate reaction in all cases. In four instances the accelerated reaction is also present, occurring for the first time when 21 days had elapsed between the first and the second injections. The *Second Division* with 11 cases shows the immediate reaction absent once and the accelerated reaction absent thrice. The *Third Division* with 19 cases shows the accelerated reaction absent once; the immediate reaction present on two occasions and certain cases doubtful. The divisions presented are not mutually exclusive nor are they precisely the divisions selected by the authors themselves, but they are based on the salient character of the reaction as defined by the interval between the first and second injections.

The first division begins with a minimum of twelve days' interval between the first and the second injections: with a shorter interval the result is negative: six cases injected at an interval of one day to six exhibited no reaction (p. 84). As with guinea-pigs, so with man, the interval between the injections of serum in the opinion of von Pirquet and Schick has an important influence in determining the presence and quality of the ensuing reaction. In the case of the immediate reaction in particular the details for guinea-pigs and for man show much agreement. The interval between injections for guinea-pigs is stated by Otto to be not less than two weeks, and up to two or three months. The interval for man is placed by von Pirquet and Schick between 12 days and six months: after this period the immediate reaction is rare. These authors agree with Otto in the view that the serum reaction has no essential relation to precipitins. In some of their instances precipitins were absent: in others they appeared at a different time from the serum reaction. Von Pirquet and Schick, in opposition to Otto and to Rosenau and Anderson, state that a large dose of serum at the first injection favours the immediate reaction.

Zucker (1905), in a series of 2323 cases, describes 24 which received serum for diphtheria on two or more occasions. He expresses the opinion that, whatsoever may be suggested by experiments on animals, the cases of his narrative offer no clinical indication of danger by a second or a third administration of antidiphtherial serum.

It is apparent from the observations cited that there occur in the human subject after two or more injections of horse serum, symptoms which, though much less severe, are comparable with the phenomena of supersensitisation induced in guinea-pigs by similar treatment with the serum of animals of a different species.

#### *Experience in Belvidere.*

The persons who form the material of the Belvidere record were diphtheria patients unless otherwise described. A minority were contacts either with diphtheria or with bubonic plague. Serum administration was subcutaneous in most cases: in fewer instances the intravenous method was employed. The period included, beginning on 1st June 1901 and ending on 31st May 1906, shows a total of 168

persons who received injections of horse serum on more than one occasion. This total figure falls to 135 by the deduction of 33 cases of diphtheria which died within six days of admission, and which both in point of time and of severity of attack excluded the serum reaction. The 135 cases are thus constituted :—

Two injections	...	...	...	115
Three injections	...	...	...	18
Four injections	...	...	...	1
Five injections	...	...	...	<u>1</u>
				135

It is proposed to collate the above figures with a *General Return* of Belvidere cases injected with horse serum irrespective of the number of times during the approximately corresponding hospital period from 1st June 1901 to 30th September 1905. This General Return, which was prepared for a purpose unconnected with this note by the Superintendent (Brownlee, 1906) of these hospitals, yields 474 cases in all. The usefulness of the comparison resides in the circumstance that the same clinical material and the same sera are handled in both records.

*Section A. Two Injections. Rash frequency.*

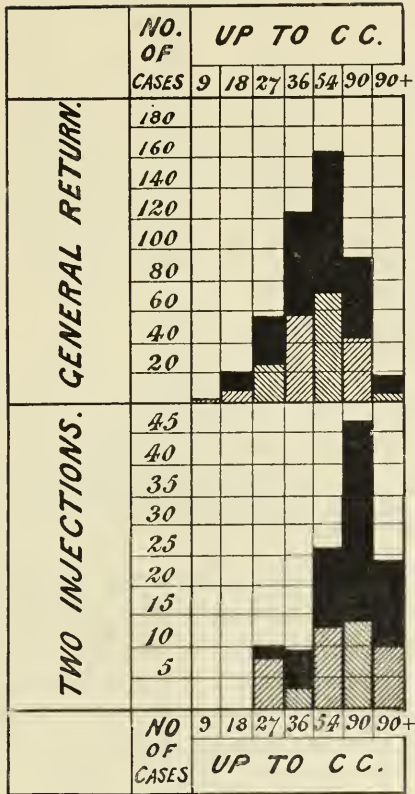
When the general return as shown in diagram A and table I. is compared with the twice injected in respect of the quantities of serum

TABLE I. *Number of Cases.*

General Return	Up to 9 c.c.	Rash 1	No rash 1	Total 2
"	18	12	8	20
"	27	29	26	55
"	36	64	58	122
"	54	89	74	163
"	90	52	42	94
"	90+	12	6	18
Total		259	215	474
Two Injections	9	—	—	—
"	18	—	—	—
"	27	2	8	10
"	36	6	3	9
"	54	13	13	26
"	90	33	14	47
"	90+	13	10	23
Total		67	48	115



Diagram A.



given and without reference to the interval between administrations, it will be observed that the twice injected exhibit a higher dosage record than the cases of the general return. Evidence however of a correspondingly varying rash incidence is not provided under this section. The general return by table II. (p. 42) has a rash frequency of 54.6%: the twice injected register a rash in 58.3% of cases. The difference is not such as to form a basis for deduction.

With respect to rash frequency in the separate dosage groups the general return by table II. indicates a constant percentage from the group of 27 c.c. to the group of 90 c.c. where the numbers of cases are large enough to have significance. The figures of the other groups are too slight to found upon. A constant rash frequency with increasing dosage, which is contrary to the sense of most records, merits a reference in this place.

TABLE II. *Percentage.*

	Up to	Rash	No rash	Total
General Return	9 c.c.	50	50	100
„	18	60	40	100
„	27	52.7	47.3	100
„	36	52.5	47.5	100
„	54	54.6	45.4	100
„	90	55.3	44.7	100
„	90+	66.6	33.3	100
Total		54.6	45.4	100
Two Injections	9	—	—	—
„	18	—	—	—
„	27	20	80	100
„	36	66.6	33.3	100
„	54	50	50	100
„	90	70.2	29.8	100
„	90+	56.5	43.5	100
Total		58.3	41.7	100

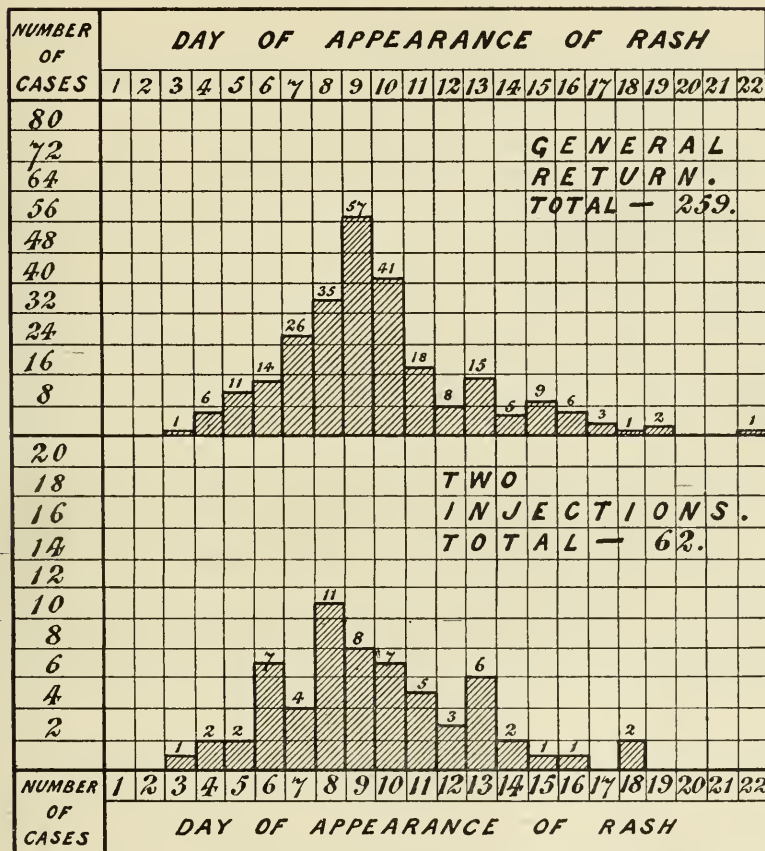
The twice injected on the other hand, fluctuating in the lower groups where the numbers are inconsiderable, present the relatively high rash frequency of 65.7 % in the 90 and 90+ groups together. The detail seems worthy of note. There is however no clear indication from the figures in general that a repeated dose of horse serum, without reference to the interval between injections, has an effect on rash frequency.

*Section B. Two injections. Day of appearance of rash.*

The time which elapsed between the injection of serum and the onset of rash in the general return is illustrated in the upper part of diagram B. The range of the incubation period is considerable, extending from the third day to the twenty second; but the majority of the rashes, 191 out of 259, have appeared by the end of the 10th day. A ten day interval thus seems a practical limit which may be applied to the twice injected cases in order to distinguish the influence of the first and second doses of serum respectively in producing rash. For the purpose of this section therefore the cases which received two injections are divided into two classes, first, cases which received the second injection of serum before the end of ten days counted from the first

injection, and, second, cases which received the second injection of serum after that period of time had elapsed.

*Diagram B: up to ten days' interval.*



When the general return is compared with the twice injected up to ten days' interval as indicated in diagram B it is noted that under the general return the maximum number of rashes, 57 in a total of 259, is recorded on the 9th day. Among the twice injected, on the other hand, up to ten days' interval, the 8th day has the maximum record, 11 in a total of 62. The crude maximum of the twice injected is thus earlier by one day. But the means of the two series are similar. The mean of the general return is 9.5; the twice injected up to ten days' interval

have a mean of 9.4. The above considerations furnish no evidence that a repeated dose of horse serum accelerates the serum rash, when the interval between the first and second administrations is up to and not exceeding 10 days.

TABLE III. *Number of Cases up to ten days' interval.*

Days between S <sub>1</sub> and S <sub>2</sub>	Day of appearance of Rash																		Total	Serial Number
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
$\frac{1}{2}$	S <sub>1</sub> S <sub>2</sub>			1		2		1		3			2	1				1	11	
1	S <sub>1</sub>	S <sub>2</sub>	1		1	3	1	4	3	2	2	1	2					1	21	
2	S <sub>1</sub>		S <sub>2</sub>	1	1	1	1	3	4		1	1				1			14	1
3	S <sub>1</sub>			S <sub>2</sub>			1	1	1	2		1		1	1				8	
4	S <sub>1</sub>				S <sub>2</sub>		1												1	
5	S <sub>1</sub>					S <sub>2</sub> 1		1											2	2, 3
6	S <sub>1</sub>						S <sub>2</sub>				1								1	
7	S <sub>1</sub>							S <sub>2</sub> 1											1	4
8	S <sub>1</sub>																		—	
9	S <sub>1</sub>									S <sub>2</sub>	1								1	
10	S <sub>1</sub>										S <sub>2</sub>	1	1						2	5
Total			1	2	2	7	4	11	8	7	5	4	5	2	1	1		2	62	

S<sub>1</sub>=first injection of serum. S<sub>2</sub>=second injection of serum.

The total of table III.=62, and the total of table IV.=5, are together equal to 67, the total number of cases in table I. which showed a rash with two injections.

The data which form the basis of the lower part of diagram B are detailed in table III. which shows for each case the day of appearance of the rash with reference to the interval of time between the injections of serum.

Although it has been stated above that the repeated injection of horse serum is not proved to accelerate the serum reaction when the interval between the injections of serum is ten days or a shorter period, it appears credible by reference to table III. that the administration of the second charge of serum towards the end of the incubation period of the first charge may suffice on occasion to determine the manifestation of a rash which would otherwise have remained undeveloped. The arrangement of the table furnishes graphic support for this suggestion. It will be observed that the line of second doses of serum in the table, receding from the line of first doses, excludes all rashes from the triangular area which is bounded by these lines and the base line. Case 3, which places its second injection in the first half of the latent period and which will be referred to immediately, is the only exception to this rule.

The suggestion is further sustained by the following consideration. In 7 of the 115 cases of table I. the second injection of serum took place in the latter half of the latent period, that is to say with 6 to 10 days' interval between the first and the second injections of serum. In two of these instances the rash failed to appear: five cases were positive as noted in table III. Though the numbers are slight the rash frequency, according to the standard of the cases reported in this paper, is high, and a rash when present invariably occurred after the second administration of serum.

Certain cases in table III. which are marked with a serial number may be briefly detailed.

*Case 1.* Ref. VI. 175, age  $3\frac{1}{2}$ . Second injection of serum on 3rd day after first injection. Two rashes. First rash, marked in table, on 7th day from first injection of serum; urticarial and general. Duration 15 days with an interval. Second rash, not marked on table, on 34th day from first injection of serum; erythematous, mild. Duration 3 days. Considered as *without reference to repeated injection*.

*Case 2.* Ref. VII. 52, age 4. Second injection of serum on 6th day from first injection. Rash on same day as second injection, and immediately following second injection; urticarial, vivid. Duration five days. Suggested *determination of rash* by second injection of serum, in the sense that the rash in the absence of the second injection of serum might possibly have remained undeveloped.

*Case 3.* Ref. II. 151, age  $1\frac{1}{2}$ . Second injection on 6th day after first injection. First rash, not marked on table, on 4th day after first injection and before



second injection of serum. Erythematous, mild. Duration 1 day. Second rash, marked on table, on 3rd day from second injection of serum; urticarial, general, vivid. Duration 5 days. *Accelerated reaction.*

*Case 4.* Ref. VII. 38, age  $2\frac{1}{2}$ . Second injection of serum on 8th day after first injection. Rash on same day as second injection, and immediately following second injection. Urticarial, general. Duration 1 day. Suggested *determination of rash* by second injection of serum.

*Case 5.* Ref. Plague G, age 35. Second injection on 11th day. A plague contact. Yersin's serum 10 c.c. on both occasions. After first injection of serum, no symptoms. On the day following second injection, erythema and oedema of arm round puncture. *Immediate reaction.*

These five cases show the accelerated reaction once, the immediate reaction once, determination so-called in two instances, and one negative record.

TABLE IV. *Number of Cases over ten days' interval.*

Days between $S_1$ and $S_2$	Day of appearance of Rash										Total	Serial Number
	1	25	38	39	241	245	481	488	1001	1009		
24	$S_1$	$S_2$									—	6
37	$S_1$		$S_2$	1							1	7
240	$S_1$				$S_2$	1					1	8
480	$S_1$						$S_2$	1			1	9
1000	$S_1$								$S_2$	1	1	10
Total											4	

$S_1$ =first injection of serum.  $S_2$ =second injection of serum.

The five cases of table IV. are also of the twice injected class, but they differ from the cases of table III. in that they received their second injection of serum after the lapse of a period of more than ten days counted from the first injection. Three cases of the five were without symptoms of supersensitisation. One case at 37 days' interval exhibited the immediate reaction. One case at 240 days' interval showed the



accelerated reaction. It is indicated by the series in table IV. that the administration of the second of two injections of antidiphtherial serum after the close of the incubation period of the first, does not infallibly accelerate the rash or induce other signs of supersensitisation.

The five cases of table IV. may be shortly recorded in this place under their serial numbers.

*Case 6.* Ref. XI. 10, age 6. Second injection of serum on 25th day after first injection. After first injection, rash on 14th day. After second injection, rash failed. Reaction of supersensitisation *absent*.

*Case 7.* Ref. I. 194, age 6. Second injection on 38th day after first injection. Two rashes. First rash, not marked on table, on 7th day from first injection. Urticarial, general. Temperature 99·4°. Duration 5 days. Second rash on day after second injection. Erythematous, local. Temperature normal. Duration one day. *Immediate reaction*.

*Case 8.* Ref. III. 1, age 3. Second injection on 241st day after first injection. Two rashes. First rash, not marked on table, on 10th day from first injection; urticarial, mild. Temperature normal. Duration 5 days. Second rash on 5th day from second injection; urticarial, severe. Temperature 102·4°. Duration 11 days with an interval. *Accelerated reaction* with reinforcement.

*Case 9.* Ref. I. 1, age 4½. Two attacks of diphtheria. Second injection on 481st day from first injection. Rash not recorded after first injection. Rash on 8th day from second injection; urticarial. Temperature 99·4°. Duration not obtained. Reaction of supersensitisation *absent*.

*Case 10.* Ref. Dr L, age 32. Second injection on 1001st day after first injection. First injection, 10 c.c. Yersin's plague serum for contact with plague. Second injection, 84 c.c. antidiphtheritic serum for diphtheria. After first injection no reaction. After second injection rash on 9th day; erythematous, local. Duration 2 days. Reaction of supersensitisation *absent*.

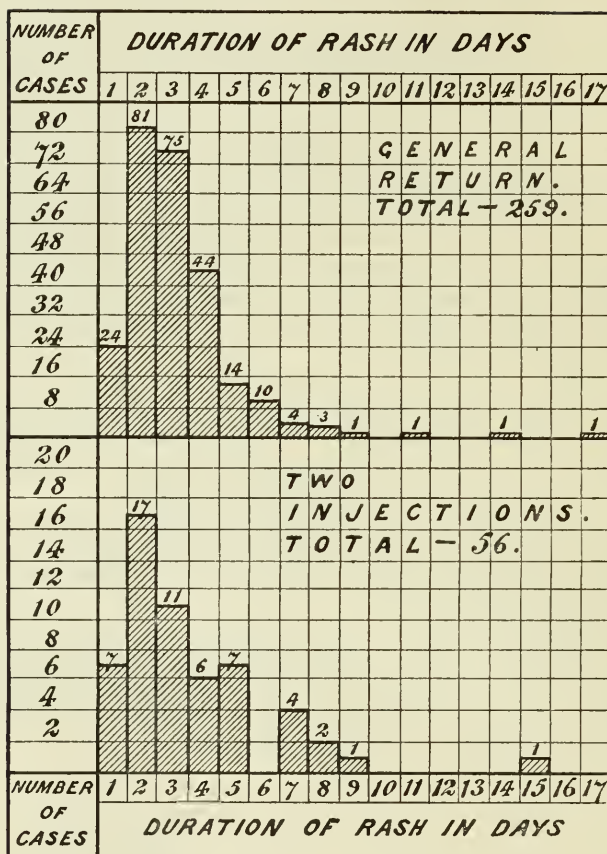
### *Section C. Two injections. Duration of rash.*

When the twice injected up to 10 days' interval, as in diagram C, are compared with the general return in respect of the duration of rash, the periods of 2 and of 3 days are observed to be the most common periods of duration in either class; and in general the two curves are similar. The mean of the general return is 3·3 days; the mean of the twice injected is 3·5 days. There is no proof that two injections under the conditions of this diagram influence the duration of the rash.

The details of the twice injected over ten days' interval may be briefly stated. The rash of case 6 lasted three days: of case 7, one day: of case 8, eleven days: of case 9, an unrecorded time: and of case 10, two days. The periods of duration vary to such an extent that no deduction seems possible.

### *Supersensitisation*

*Diagram C: up to ten days' interval.*



The total for two injections of diagram C is less than the total for two injections of diagram B owing to the omission from diagram C of certain cases regarding the duration of whose rashes in formation was not obtained.

*Section D. Three injections. Rash frequency and the like.*

From table V which states the total quantities of serum received by the thrice injected it is apparent that this class represents a higher dosage rate than the general return or the twice injected. While the general return over 54 c.c. shows 112 cases in 474 a percentage of 23.6, and the twice injected in the same dosage groups have 70 cases in 115,

a percentage of 60·8; the thrice injected cases without exception are seen to have received more than 54 c.c. of serum. The thrice injected, which number 18, exhibit 13 rashes and 5 failures. The five cases which furnished no reaction had intervals of from 3 to 17 days between the first and the third administrations of serum.

TABLE V. *Dosage Rate.*

Up to	Rash	No rash	Total
54 c.c.	—	—	—
90	7	3	10
150	6	1	7
192	—	1	1
Total	13	5	18

The thrice injected are compared with other groups as regards rash frequency in table VI. According to this table the thrice injected have 72·2 % of rashes, a higher ratio than the preceding groups. The thrice injected are few, but an association between dosage and rash frequency is suggested.

TABLE VI. *Percentage comparison of thrice injected with the General Return and the twice injected.*

	Rash	No rash
General Return	54·6	45·4
Two Injections	58·3	41·7
Three Injections	72·2	27·8

Details regarding the thrice injected are presented in tabular form in table VII. The group may appropriately be divided into two series according as the last administration of serum falls within or without the ten day period to which reference was made above.

*Series I.* Cases 11 to 17 show the third injection of serum within the latent period: that is to say, not later than the 11th day. They do not differ essentially from cases of the general return. The severe reaction of *case 11*, ref. i. 149, age 4, on the 14th day from the first injection, is possibly worthy of remark: and notice may be taken of the late rash of *case 17*, ref. xi. 41, age 2, which appeared on the 25th day. Immediate and accelerated reactions are alike absent in this series.

*Series II.* Cases 18 to 23 show the third injection of serum without the ten days' interval.

*Case 18.* Ref. vi. 138, age 3. Total dose 135 c.c. The third injection of serum and rash both on 13th day, but rash preceding the third injection of serum. Possibly *accentuated reaction* in the sense that the reaction was more severe than might credibly have been the case in the absence of the third injection of serum.

TABLE VII. *Details.*

Serial Number	Day of administration of				Days between $S_1$ and $S_3$	Day of appearance of rash	Duration of rash in days	Temperature	Quality of reaction
	$S_1$	$S_2$	$S_3$	$S_3$					
11	1	2	4		3	14	—	105·6°	Macular: vivid: general.
12	1	1	4		3½	9	3	99°	Urticarial: general.
13	1	2	6		5	8	5	103°	Urticarial: vivid: general.
14	1	2	8		7	9	2	Normal	Urticarial: moderate.
15	1	2	8		7	10	10	100°	Urticarial: recurrent.
16	1	3	10		9	15	2	Normal	Scarlatiniform.
17	1	8	11		10	25	2	102°	Morbiliform.
18	1	3	13		12	13	2	104·8°	Morbiliform: very vivid.
19	1	25	26		25	27	5	102°	Urticarial: general.
20	1	35	36		35	42	1	101·4°	Urticarial: local.
21	1	15	43		42	8	5	99°	Urticarial: general.
						45	1	Normal	Urticarial: local.
22	1	556	558		557	559	4	100·4°	Urticarial: general: very vivid.
23	1	1365	1838		1837	10	2	Normal	Urticarial: with articular pain.
						1372	10	Normal	Urticarial: severe.
						1846	1	Normal	Urticarial: extremely severe.

$S_1$  = first injection of serum.  $S_2$  = second injection of serum.  $S_3$  = third injection of serum.

*Case 19.* Ref. II. 149, age 4. Total dose 126 c.c. Second injection of serum on 25th day. Third injection of serum on 26th day. Rash on 27th day. Probably *immediate reaction* after second injection.

*Case 20.* Ref. VII. 86, age  $1\frac{2}{12}$ . Total dose 72 c.c. Second injection on 35th day for a recrudescence of diphtheria. Third injection of serum on 36th day. Second injection followed in 7 days by rash of 1 day's duration. *Accelerated reaction* after the second injection suggested by brevity of rash.

*Case 21.* Ref. VII. 102, age 3. First injection 36 c.c. followed by first rash on 8th day. Second injection 27 c.c., for recrudescence of diphtheria, on 15th day, not followed by a rash. Third injection 27 c.c., for a further recrudescence, on 43rd day, followed in 2 days by second rash. First rash general, lasting 5 days. Second rash local, lasting one day. Second rash shows *accelerated reaction* in respect of early onset after third injection and in respect of briefer duration than the first rash.

*Case 22.* Ref. II. 50, age  $2\frac{1}{2}$ . First injection 12 c.c. prophylactic of diphtheria. No record of rash. Second injection 36 c.c. on 556th day. Third injection 21 c.c. on 558th day. Second injection followed in 3 days by rash, severe and of 4 days' duration. *Accelerated reaction* after second injection.

*Case 23.* Ref. Dr B. age 38. First injection Yersin's serum 10 c.c., prophylactic of plague, followed in ten days by first serum rash, urticarial with articular pain, of 2 days' duration. Second injection antidiphtheritic serum 18 c.c., for diphtheria, on 1365th day, followed in 7 days by the second serum rash, a severe urticaria of 10 days' duration. The second serum rash had thus a shorter latent period and a more rigorous course than the first serum rash. On 1824th day onset of influenza. On 1832nd day an urticarial rash not associated with serum, of moderate intensity and lasting 5 days. The third injection 27 c.c. antidiphtheritic serum for another attack of diphtheria, on the 1838th day, followed in 8 days by the third serum rash, an urticarial rash of extreme severity and of 1 day's duration. The third serum rash had a longer latent period than the second serum rash, a shorter than the first serum rash. Symptoms attending the third serum rash had violence and brief duration of *accelerated reaction*. The three rashes which ensued on the three administrations of serum show a progressive increase in severity. The occurrence of the rash not associated with serum suggests a constitutional facility as regards urticaria.

Series I. of this group, in which the third injection of serum falls within the latent period shows reactions which may be regarded as normal. Series II. of this group in which the third injection of serum falls without the latent period, has in each case a serum reaction which differs from the result of a single administration.



*Section E. More than three injections.*

This group contains two cases only.

*Case 24.* Ref. II. 104, age 6. 4 injections. The fourth injection of serum on 7th day. Total dose 198 c.c. Severe diphtheria. No rash. Death on 7th day from first injection of serum.

*Case 25.* Ref. IX. 126, age 7. 5 injections. The fifth injection of serum on 7th day. Total dose 222 c.c. Severe diphtheria. No rash. Recovery.

In each instance the last dose of serum fell within the latent period of the first, and the absence of exceptional reactions was to be expected.

*General Considerations.*

It is now proposed to compare the Belvidere cases with the cases recorded by von Pirquet and Schick: to state in a summary form the conclusions which the Belvidere observations suggest; and thereafter to note certain general aspects of the phenomena under discussion.

Experience at Belvidere is in accord for the most part with the results tabulated by von Pirquet and Schick (1905, p. 89) for double injection. The comparison may be extended also to the thrice injected cases of this paper.

The first division in the table of von Pirquet and Schick, which shows immediate reaction only, with a period of 12 to 50 days between the injections of serum, corresponds with cases 5, 7, and 19. Case 5 exhibits the immediate reaction with an interval of 10 days between the first and the second injections of serum, that is to say, with a shorter interval than is tabulated by von Pirquet and Schick for cases which furnished an unquestioned reaction. In this connection it may be borne in mind that, in the experience of the Belvidere Hospitals, the average incubation period of plague serum has been shorter by two days than the average incubation period of antidiphtheritic serum. Case 7 shows an immediate reaction when 37 days intervened between injections, and case 19 has an immediate reaction with a period of 24 days between the first and the second administrations of serum, a third injection on the day following the second injection being neglected. In Cases 7 and 19, therefore, the interval between the injections of serum falls within the 12 to 50 day period of von Pirquet and Schick's first division.

The second division of von Pirquet and Schick which showed both immediate reaction and accelerated reaction, with an interval



of two to six months between injections, is not represented in this record.

The third division of von Pirquet and Schick which displayed the accelerated reaction only, and in which a period of from seven months to seven and a half years elapsed between the two injections of serum, may be compared with the following cases.

Case 3, which showed a first rash on the 4th day from the first injection of serum, had the second injection on the 6th day from the first injection. The second injection was followed in two days by the second rash, an example of the accelerated reaction. A five days' interval between two injections which induced an accelerated reaction is a lower record than is furnished by von Pirquet and Schick's table, where the accelerated reaction—with an immediate reaction preceding—appears for the first time with a period of 21 days between two injections of serum.

In case 20 the accelerated reaction occurred with an interval of 34 days between the first and second injections of serum, a third injection on the day following the second injection being neglected. Case 21, which exhibited a first rash on the 8th day from the first injection of serum, had on the 15th day a second injection which was not followed by a rash. The third injection in this case, which was administered on the 43rd day from the first injection, induced a rash which had the accelerated character. Cases 20 and 21, therefore, with periods of 34 and 42 days respectively between the first and the effective subsequent injection of serum, have shorter intervals between injections than the cases of von Pirquet and Schick's third division,—whose minimum interval between injections is seven months,—and may be compared with instances in their first or second divisions, where the accelerated reaction occurs with or without a preceding immediate reaction, and where the interval between injections of serum is up to and not exceeding six months.

In case 8 the accelerated reaction was noted, the interval between the first and the second injections of serum being 240 days. Case 22 exhibited the accelerated reaction with a period of 555 days between the first and the second injections, a third injection two days after the second injection being neglected. In case 23, 1364 days intervened between the first and the second injections, and 1837 days between the first injection and the third. In this case an accelerated reaction was observed both after the second and after the third injections. Cases 8, 22 and 23, therefore, as regards length of interval between injections,

are within the period of von Pirquet and Schick's third division, which extends from seven months to seven and a half years.

Certain statements are suggested by a view of the Belvidere cases.

*The Twice Injected.* There is no evidence that the twice injected have a higher *rash frequency* than the general return.

There is no evidence that the *latent period* among the twice injected, when the interval between the first and second injections is up to 10 days, differs from the latent period of the general return. It is credible that the administration of the second injection of serum in the closing days of the latent period of the first should on occasion make manifest a rash which would otherwise have failed to appear. The administration of the second of two injections, after the termination of the latent period of the first, may curtail the latent period of the second injection, but is not infallible in this respect.

There is no evidence that the rash among the twice injected differs in *duration* from the rash of the general return.

*The Thrice Injected.* The thrice injected show a higher *rash frequency* than the general return or the twice injected. This is coincident, and probably associated with a relatively higher dosage rate.

There is no evidence that the *latent period* among the thrice injected, when the interval between the first and the third injections is up to and not exceeding 10 days, differs from the latent period of the general return. When the interval between the first and the third injections is over 10 days, the thrice injected consistently show a modification of the ensuing serum reaction with a reduction in the length of the corresponding latent period.

From the foregoing statements it emerges that the interval of time between the first and the second injections of serum is a primary factor in determining the abnormal reaction which has been regarded as evidence of supersensitisation. The length of the interval is at least more essential to the phenomenon than the administration of serum in large doses. Von Pirquet and Schick concur in this opinion, even although they differ from other observers in considering large doses more effective than small.

It will be recalled that Wright (1903) has drawn attention to processes in immunization where the interval between administrations of the active substance has an important influence. He indicates, for example, that the inoculation of vaccine is followed in the first place

by a diminution, and in the second place by an increase in the bactericidal value of the blood. The fall and the subsequent rise he terms the negative and the positive phases of the immunity curve. He states that a cumulative negative phase is produced by injection of the immunizing agent during the negative phase of the preceding administration, and that a cumulative effect is most to be feared when the amount injected is excessive. Supersensitisation, however, does not appear as a cumulative result of excessive dosage. Otto (p. 16) found small quantities of serum more disastrous to guinea-pigs than large: he is followed by Rosenau and Anderson (1906), who procured supersensitisation of a guinea-pig by  $\frac{1}{1,000,000}$  c.c. of horse serum, a dose whose minuteness recalls the report of von Behring and Kitashima (1901, p. 162), that the death of an animal was caused by successive injections of diphtheria toxin which amounted in sum to  $\frac{1}{400}$ th of the minimum lethal dose. In view of such data as these, the cumulative theory seems inapplicable to the phenomena of supersensitisation.

Certain other theories which are mentioned by authors (see von Pirquet and Schick, 1906) may be noted in this place. Courmont's absorption theory would explain supersensitisation on the hypothesis that the first injection of the active material leads to the absorption of a natural protective substance, and that the animal is thus left defenceless against the second injection. Bail has expressed the view that death, after two injections of serum, is associated with the production of a substance which impedes the activity of leucocytes. Richet has suggested that the phenomena in question are due to the presence in the serum injected of two separate bodies, of which one causes immunity and the other supersensitisation. It is a disadvantage of the theories of Courmont, Bail and Richet that they are formulated without special regard to the influence exerted by the interval of time between the separate administrations of serum.

The endotoxin theory of Wolff (1904) has reference to the effects produced in the animal tissues by the liberation of poisons contained within the bodies of bacteria, whose external covering has been penetrated or dissolved as a result of defensive measures adopted by the animal concerned. In the opinion of Wolff there is no immunity to foreign albumens of the endotoxin class: but, even if it be the case that the action of endotoxins furnishes an adequate explanation of abnormal phenomena which may follow the introduction into an animal of bacteria whose hurtful effect is exerted after the destruction of their

outer covering, the theory does not seem applicable to extraneous blood sera, whose constituents are free at the time of injection.

The precipitin theory is discussed by Otto, by Rosenau and Anderson, and by von Pirquet and Schick. According to these observers the presence of precipitins in the blood of animals or human beings, whether normal or supersensitized, did not synchronize with the serum reaction. In some cases the serum reaction was the first to appear: in others, precipitins were earlier recorded. In some cases, precipitins were present, and the serum reaction was absent: in others, the reverse occurred. Nevertheless, it is noteworthy that precipitin formation and the serum reaction, whether typical or abnormal, tend to be manifest side by side, as if they resulted by the action of allied substances.

That these substances are probably antibodies is suggested by the following considerations. In the case of *precipitin*, it was shown by von Dungern (1903), who experimented on rabbits with the plasma of the crustacean *Maja squinado*, that the latent period before the appearance of precipitin constantly approximated to six days: similarly, the *serum* reaction, as is matter of common knowledge, develops after an interval of incubation. Further, in the case of *precipitin* it is recorded by von Dungern (*ibid.*), in the first place, that rabbits immunized to the plasma of *Maja squinado* show precipitin after a shorter latent period than normal rabbits; and, in the second place, that repeated injection of rabbits with *maja* plasma within the latent period of six days does not accelerate the appearance of precipitin: similarly, in the case of *serum* phenomena it is the sense of this note and of the works quoted, in the first place, that the sensitized organism furnishes an earlier reaction than the organism not previously treated, and, in the second place, that the administration of succeeding injections of serum within the incubation period of the first fails to accelerate the ensuing rash. The serum reaction not less than precipitin formation is in accord with the law of the latent period, which concerns the development of antibodies. The occurrence of the serum reaction after the injection of extraneous sera may thus be regarded as due to the elaboration of antibodies in the organism, by substances in the injected material which are allied but not identical with the substances which induce precipitin formation.

The *normal* serum reaction, in accordance with this theory, will be understood in the sense that the injection of extraneous serum leads after a quiescent interval to the elaboration of antibody, and that by



the interaction of a substance contained in the serum injected and of the antibody which it originates, there is formed a toxic product whose presence is made known by the so-called serum reaction. It is credible from analogy that the antibody in question is gradually brought into existence: it may be, therefore, that the antibody-producing substance, uniting with nascent antibody, gradually frees the toxic product, which the organism under these circumstances is able gradually to eliminate. Or, it is possible that the toxic product evokes a secondary antibody, which combines with the toxic product, controls its effects, and ultimately brings the visible reaction to an end. The incubation period of this secondary antibody must be regarded as shorter than the incubation period of the primary antibody.

In the case of the *abnormal* serum phenomena which mark the supersensitive state, there is support for the opinion that the immediate and the accelerated forms are to be ascribed to different causes.

The immediate reaction is exempt from the law of the latent period. If the antibody has a part in the phenomenon, it is not necessary at least that time should elapse in order that the antibody may be prepared. On the contrary, it is to be supposed that the antibody to the first injection of serum is still present when the second injection is given. The antibody-producing substance of the second injection of serum, and the antibody already produced by the first injection, come in contact, under these circumstances, without delay: their union is rapid: the whole charge of the poisonous substance is freed in a brief period, and the toxic symptoms tend to be sudden and severe. If the theory of a secondary antibody is accepted as relevant in respect of the normal serum reaction, the special characters of the immediate form of the abnormal reaction will be explained on the view that, while the primary antibody produced by the first injection of serum persists at the time of the second injection of serum, the secondary antibody evoked by the toxic substance which resulted from the combination of the first injection of serum with the primary antibody has already vanished from the organism. The disappearance of the secondary antibody within a limit of days may be supported on the analogy of relapsing fever, in which Löwenthal<sup>(11)</sup> records a rapid fall of the bactericidal content of the serum during the apyrexial interval of the disease. When, therefore, the antibody-producing substance of the second injection of serum reacts with the primary antibody produced in the organism by the first injection of serum, the abruptly liberated toxic product of this reaction exerts its hurtful influence unchecked

until sufficient time has elapsed to admit of the preparation anew of a secondary antibody to control its effects. When the immediate reaction is absent, it is open to belief that the primary antibody has disappeared, or was present in too small a measure to induce an appreciable result.

With regard to the general mechanism of the processes considered, it may be, in terms of Ehrlich's hypothesis<sup>(9)</sup>, that the second administration of serum takes place at a time when specific side chains, elicited by the first injection of serum, are still in course of formation, and that the higher affinity of the so-called sessile receptors for antibody-producing substances contained in the second injection is responsible for the distinctive features of the immediate serum reaction.

The accelerated reaction differs from the immediate reaction in observing the law of the latent period. The accelerated reaction occurs with or without a preceding immediate reaction, and may be regarded as independent of the presence of antibody in the animal at the time of the second or succeeding injection of which it is the result. Rather it is to be attributed to a tissue modification, in virtue of which there is developed a more rapid cellular response to the stimulus of material present in the serum injected. The accelerated reaction is evidence of the acquisition on the part of the animal of a faculty which is normally useful. Certain parasitic diseases in nature, which obtain a foothold in the animal tissues, attack these through the natural channels of access, and by means of small quantities of bacteria, living organisms which are capable of growth and reproduction. The multiplication of the infecting bacteria in such cases may be taken generally as a sign that their assault is being attended by a measure of success. An animal, therefore, which develops the capacity of rapidly preparing antibodies to restrict the proliferation of such noxious agents, when introduced through natural channels, has achieved an immunity to parasitic diseases of a certain natural type.

But, if the active principle is introduced into the system neither through the customary channels nor under the form of a micro-organism, whose power for mischief depends on its liberty to grow and multiply, the procedure is out of accord with the course of nature, and the defensive powers of the animal, adapted to cope with natural infections, are somewhat at fault in their method of dealing with the artificial invasion. It is true that a mechanism of the protective class is stimulated by repeated injections of extraneous serum into an animal;



but the protective value of the mechanism in question resides in the circumstance that it is suited to check the elaboration of toxic products within the system; it is not adjusted to neutralize or counteract a definite dose of such a substance as blood serum, which is not capable of numerical increase. Extraneous sera appear to belong to an order of substances which effect immunization, not by inducing insusceptibility of the tissue cells, but by means of an accelerated reaction, which may thus be regarded as the expression of a misdirected defence, a formal but useless immunity.

The accelerated reaction conforms to the theory that two antibodies are concerned in serum phenomena. To the more rapid formation of the primary antibody, the reduction of the latent period may be looked on as due: to the speedier preparation of the secondary antibody the accelerated reaction owes its commonly briefer course.

Other aspects of the serum reaction which are discussed or disputed by authors have been noticed in their place. The capricious variability of the signs under conditions apparently similar, the relative effectiveness of large and of small first doses, the comparative mildness in man of the immediate reaction, the apparently exclusive incidence in man of the accelerated reaction, and the possible sensitizing influence on man of a diet of horse-flesh are problems of interest, but their solution is not promoted by the data of this paper. The relationship, also, of intravenous injection to supersensitisation remains open to discussion.

From the practical standpoint, however, it is apparent that the facts detailed are consistent with the view that repeated injections of horse serum induce symptoms of supersensitisation in man; but it is also apparent that the same facts lend no countenance to the suggestion that the death of persons suffering from diphtheria is to be apprehended as the result of repeated injections of antidiphtherial serum. Experiments on animals may favour the opinion that such a disaster is a speculative possibility in the case of the human subject, but there is no reported evidence to the effect that it is so imminent a danger as to excuse a restricted application of the antitoxin treatment.

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## EXAMPLES OF THE IMMEDIATE AND OF THE ACCELERATED REACTION FOLLOWING TWO INJECTIONS OF ANTIDIPHThERIAL SERUM.

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SINCE the close of the period which furnished the data for the preceding paper regarding the effects of repeated injections of horse serum, two cases have been observed in the practice of Belvidere Fever Hospital which have special bearing on the subject of abnormal rashes after suitably repeated doses of antidiphtherial serum.

The first of the two cases, *David W.*, age 1 year and 4 months (Ref. XII. 115), was admitted to hospital 5. x. 1906, suffering from diphtheria. On his admission he received 54 c.c. of antidiphtherial serum by subcutaneous abdominal injection. After an interval of 15 days he showed a general and somewhat profuse morbilliform rash of two days' duration. His highest recorded temperature during the rash was 102° Fahr.

When 21 days had elapsed from the first injection of serum a recrudescence of diphtheria occurred, and the patient received a second injection of antidiphtherial serum, again into the subcutaneous tissues of the abdomen, estimated on this occasion at 18 c.c. Within half an hour of the second injection of serum an urticarial rash was visible on the skin of the abdomen. In six hours the rash had become vivid and general, extending over the whole surface of the body. Six hours later its brightness had begun to fade, and 21 hours after the second injection a faint suffusion in the area of puncture alone was seen. Twenty-four hours after the injection no trace of the rash remained.

Before the administration of the second injection of serum the temperature of the patient was 98·4° Fahr. Fourteen hours after injection it reached its maximum at 103°. Thirty hours after injection

99·8° was recorded, and thirty-four hours after injection the temperature had receded below the normal line to 97·6°.

In this case the second rash was perceptible within half an hour of the second injection: so brief a period forms a contrast with the fifteen days which intervened between the first injection of serum and the first rash. The second rash further differs from the first rash in having a duration of one day instead of two days.

The case now under consideration, in which the interval between injections of serum was twenty-one days, corresponds with the cases in von Pirquet and Schick's tabular statement (1905, pp. 89—90)<sup>1</sup> in which the interval recorded between injections was twelve to fifty days. In its rapid onset, appreciable severity and brief duration the second rash of this case is typical of the *immediate serum reaction*.

The second of the two cases *Janet G.* (Ref. xv. 18) was twice admitted to Belvidere Hospital suffering from diphtheria. A period of five years separated the two attacks. On both occasions she received antidiphtherial serum.

On her first admission to hospital (9. XI. 1901) in the sixth year of her age, she underwent tracheotomy, and 48 c.c. of antidiphtherial serum were administered subcutaneously. In seven days from this first injection of serum a rash appeared, urticarial at its onset, later morbilliform, lasting three days. The rash was general and very profuse. It was not attended by a higher temperature than 98·8° Fahr.

On admission to hospital five years later (31. x. 1906), and in the eleventh year of her age, the child was again suffering from diphtheria. Membrane was situated on the fauces: the attack was of moderate severity. At this time 27 c.c. of antidiphtherial serum were injected into the subcutaneous tissues of the abdomen: an interval of 1817 days divided this, the second injection, from the first. On the 5th day from the second injection of serum an urticaria appeared on the chest. On the 6th day the limbs showed a circinate erythema: the face and the hands were swelled and marked by thick-set morbilliform macules: discomfort and restlessness were extreme. On the 7th day the signs had faded in some measure: but on the 8th day urticaria was general. On the 9th day a general circinate erythema and a general urticaria were both recorded. On the 10th day the skin was no longer

<sup>1</sup> von Pirquet and Schick (1905) *Die Serumkrankheit*. Franz Deuticke, Leipzig und Wien, pp. 89—90.



abnormally coloured, but the swelling of the face persisted until the 11th day. On the 12th day the condition of the child was normal. The highest temperature ascertained during the continuance of the rash was  $100\cdot2^{\circ}$ . The duration of the rash was seven days.

The rash which followed the second injection of serum in this case may be looked on as typical of the *accelerated reaction*. The rash after the second injection was earlier in its onset by three days than the rash after the first injection. The severity of the second rash is contrasted with the comparative mildness of the rash which followed the first injection. The second rash had a longer visible course than the three days of the first rash: but its seven days' duration is less than that of a case recorded by von Pirquet and Schick (1905, p. 90 *loc. cit.*) in their table, in which the accelerated reaction lasted for eight days. As regards the interval of time between the two injections of serum, the case is within the limit of cases tabulated by von Pirquet and Schick (p. 90) which furnished an accelerated reaction, and in which the interval between injections was seven months to seven and a half years.

The case of Janet G. may almost be said to have the value of a controlled experiment in view of the fact that her sister, *Maud G.* age 5 (Ref. xv. 20), and her brother *Thomas G.* age 8 (Ref. xi. 92) were received into hospital each with a first attack of diphtheria on the same day and at the same hour as Janet G. was admitted with her second attack of diphtheria. In all three cases the faucial appearances were approximately similar: the sisters were equally affected; the membrane in the boy's throat was somewhat more extensive. In all three cases antidiphtherial serum was administered at the same time: each of the sisters received 27 c.c. whilst 36 c.c. were given to the boy.

In the case of Janet G., as stated above, there began on the 5th day a rash of much severity and of seven days' duration: in the case of Maud G. no reaction was apparent until the 9th day after injection, when a trivial urticaria of eight hours' duration appeared on the abdominal skin. In the case of Thomas G. the 12th day was reached without evidence of rash, but in the course of the 12th day the abdomen showed slight urticaria. This urticaria was intermittent, lasting for two days in all.

The rashes of the G. family thus differ from one another in accordance with the view that a previous injection of antidiphtherial serum favours an abnormally active response to a succeeding injection.

The details of the cases described are appended in tabular form.



TABLE.

Name and details:—	<i>David W.</i> , age $1\frac{1}{2}$ yrs. Two injections at interval of 21 days		<i>Janet G.</i> , age 10 at time of second injection, Two injections at interval of 1817 days		<i>Maud G.</i> , age 5. One injection	<i>Thomas G.</i> , age 8. One injection
	First injection	Second injection	First injection	Second injection	Single injection	Single injection
Injections						
Quantity of serum in- jected	54 c.c.	18 c.c.	48 c.c.	27 c.c.	27 c.c.	36 c.c.
Time be- tween in- jection and succeeding rash	15 days	$\frac{1}{2}$ hour	7 days	4 days	8 days	11 days
Duration of rash	2 days	1 day	3 days	7 days	8 hours	2 days
Highest tem- perature during rash	102°	103°	98·8°	100·2°	98°	99°
Quality of rash	Morbilliform	Urticarial	Urticarial and morbilliform	Urticarial with circinate erythema	Urticarial	Urticarial
Distribution and degree of rash	General: somewhat profuse	General: profuse	General: very profuse	General: extreme severity	Local: trivial	Local: trivial, intermittent

# ON THE RELATION OF THE ANTITOXIN TO THE GLOBULIN-CONTENT OF THE BLOOD SERUM DURING DIPHTHERIA IMMUNISATION.

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WITHIN recent years a considerable amount of attention has been directed to the subject of the relationships subsisting between the proteids of the blood serum and the antibodies elaborated therein by various methods of immunisation.

One result of such study has been to give an impetus to the more accurate differentiation of the several proteid constituents regarded as bodies possessing not only definite physical and chemical properties, but also what we, with our present knowledge, may call functional affinities.

The investigations recorded in the present paper, dealing with the quantitative relations of the serum globulins during the process of immunisation with diphtheria-toxin, were instigated by the fact that, hitherto, with the exception of the work of Hiss and Atkinson (1901) to be later referred to, only isolated observations have been made of the globulin variations in relation to potency.

It was felt that the only satisfactory method of procuring reliable data on the globulin-antitoxin question was to institute frequent quantitative estimations throughout the whole period of immunisation of individual horses or goats and not at random intervals in their history as antitoxin-producers.

For some considerable time it has been a well-established fact that antitoxin is precipitable from serum by any precipitants which throw down the globulins, but more recent research has shown that the term "globulin" comprises two or more bodies having different salt precipitation limits as well as different antitoxin-contents.

The history of this subject, dealing with the differentiation of the globulins and their relation to the antitoxic substance, is so important in connexion with the observations to be presently recorded, that brief reference must be made to the literature. The work of Marcus (1899), following at long interval after the preliminary observations of Burckhardt (1883), went to show that the hitherto accepted characteristic of serum-globulin in contradistinction to serum-albumin, viz. its insolubility in salt-free water was untenable because, after precipitation by dialysis of the typical insoluble globulin, there remained in solution a relatively much larger quantity of a proteid body, presenting no essential differences from the first either in chemical reaction, coagulation temperature, or elemental constitution.

Consequently, according to Marcus, one must either give up insolubility in water as a characteristic property of globulin or consider the soluble globulin as constituting a new proteid group.

So far, it was impossible by fractional salt precipitation to isolate bodies corresponding to these soluble and insoluble globulins.

Before Marcus' work, attempts had been made to determine the chemical nature of diphtheria-antitoxin and its relation to the serum-proteids.

Thus Aronson (1893) found that the globulin precipitated by dialysis was potent as well as the globulin-free filtrate, while Dieudonné (1897), by magnesium sulphate precipitation, obtained the greater part of the antitoxic substance in the precipitate and only a small part in the globulin-free filtrate.

Brodie (1897) was the first to show that diphtheria-antitoxin was completely precipitated from a solution by any means which removed the globulins. He also attempted a fractional precipitation of serum with ammonium sulphate up to half-saturation, thus separating four successive portions, all of which were found to contain antitoxin in equal amount. That the mode of preparation of the globulin was of importance was shown by Belfanti and Carbone (1898), who found that the antitoxin was carried down along with the globulins from ammonium or magnesium sulphate precipitation but not with the precipitate obtained by acetic acid.

In the course of an attempt to follow up Brieger and Boer's work on the preparation of a proteid-free antitoxin, Freund and Sternberg (1899) discovered that by addition of a solution of potash-alum to serum (to one volume serum, one-third volume of a 5% solution of potash-alum) all the antitoxin remained in the filtrate. In fact, by this

precipitant the albumin was removed first and a filtrate left which, according to Freund and Sternberg, contained practically all the globulin and consequently all the antitoxin. Further, by precipitating this globulin filtrate with ammonium sulphate, it was possible to obtain in small bulk the globulin-content of a large quantity of serum. No further light, however, could be thrown by these investigators on the chemical nature of antitoxin. All that could be said was that the antitoxic substance was precipitated along with the globulins.

The method of treating serum with potash-alum in order to get rid of albumin was employed by Seng (1899), who dealt with the question whether the sera of normal and immune animals presented differences in the amount and nature of their proteid constituents. The "soluble" and the "insoluble" globulin fractions were separately estimated, and the total proteid determined by Kjeldahl.

From the few isolated estimations on horses immune to diphtheria-toxin, no conclusive facts could be elicited as to the relative quantities of total globulin, albumin, and insoluble globulin. Though no quantitative differences could be found between the "soluble" globulin-content of normal and antitoxic serum, certain slight differences in coagulation temperature and specific rotation were made out. It was further determined that prolonged dialysis of the potash-alum filtrate until no reaction to chlorine, ammonia, or sulphuric acid remained, rendered only a very small fraction of the globulin insoluble, viz. 1:23 to 1:11. All the antitoxin was recoverable from the solution. These latter experiments of Seng threw light on the rather discordant results of earlier writers. Thus the result obtained by Belfanti and Carbone that the antitoxin falls out with the globulins when precipitation is effected by magnesium or ammonium sulphate, but not by acetic acid or carbon dioxide, is explained by the fact that simple neutralization of the serum by acetic acid or carbon dioxide precipitates only the "insoluble" globulin which contains none of the antitoxin. So also the statement by Dieudonné that "reines globulin" precipitated by carbon dioxide or by dialysis contained no antitoxin is explained on similar grounds.

A notable advance was made by Hiss and Atkinson (1901), who estimated the globulin-content of the serum of a large number of horses at different stages of immunisation against diphtheria-toxin. The globulin was precipitated by the magnesium sulphate method. The total proteid was obtained by heat coagulation and the albumin estimated by difference. As a rule not more than two globulin estimations were made on each horse, one before the commencement

of immunisation and the other at some stage in the process. When the potency had reached a level of 300 units or over, the globulin was invariably found to have risen, sometimes to double its original value. In one horse five globulin estimations were made during immunisation, the figures (obtained from 10 c.c. serum) being as follows:

Normal	400 units	600 units	650 units	1200 units
·3235	·4743	·5116	·5934	·8987

A progressive increase in globulin was thus evident as the potency rose. While the globulin rose the albumin fraction progressively diminished. It appeared generally that a low potency coincided with a low globulin-content, but it was impossible to take the absolute amount of globulin as an index of the antitoxin-content of the serum.

Within the last few years important contributions to our knowledge of the globulins have emanated from the Hofmeister school. Fuld and Spiro (1900) determined that by fractional precipitation of serum with ammonium sulphate two globulin fractions could be obtained, one precipitable by 28–33 % saturation and the other only by 34–46 % saturation. To the former fraction precipitable by one-third saturation, Hofmeister gave the name “euglobulin,” and to the latter precipitable only by half-saturation, the name “pseudoglobulin.” Included in the euglobulin fraction is the fibrinoglobulin, which Reye (1898) had already found to be precipitated by 21·5 % saturation with ammonium sulphate. The interesting fact was recorded that these three fractions differed with regard to their influence on milk coagulation. The fibrinoglobulin had no constant action either in producing or preventing coagulation. The euglobulin had decidedly the power of coagulating milk, while the pseudoglobulin had a more or less pronounced inhibitory action on milk coagulation.

The relation of diphtheria-antitoxin to the three globulin fractions precipitable by ammonium sulphate up to half-saturation was investigated at great length by Pick (1902), who found that neither the fibrinoglobulin nor the euglobulin of horse serum contained the antitoxin. The pseudoglobulin, on the other hand, contained all the antitoxin. On the other hand, when goat serum was salted out in the same way and the fractions tested as to their antitoxin-content, it was found that the euglobulin contained the antitoxic substance.

Freund and Joachim (1902) repeated much of Spiro's work, and were unable to identify completely the euglobulin and pseudoglobulin with the “insoluble” and “soluble” globulin obtained by dialysis,



acetic acid or sodium chloride. It was determined that both the euglobulin and the pseudoglobulin could again be split up into two portions, one of which was soluble and the other insoluble in water. They accordingly recommended the following as a more accurate classification of the globulins:

Fraction obtained by one-third saturation	$\alpha$ . Insoluble in $H_2O$ .	Paraeuglobulin.
	$\beta$ . Soluble in $H_2O$ .	Euglobulin.
Fraction obtained by half-saturation	$\alpha$ . Insoluble in $H_2O$ .	Parapseudoglobulin.
	$\beta$ . Soluble in $H_2O$ .	Pseudoglobulin.

There is no doubt that the division into euglobulin and pseudoglobulin is a more or less artificial one from the purely chemical point of view as Hammarsten maintains, but the remarkable differences exhibited by them in their capacity as antibody-carriers proclaim a real duality and render it highly essential that we should retain this somewhat arbitrary mode of separation.

Porges and Spiro (1903) admitted the correctness of Freund and Joachim's statement that the eu- and pseudoglobulin fractions did not represent exactly the sum total of the insoluble and soluble globulins respectively. In the same year Joachim (1903) recorded a few quantitative estimations of the globulin fractions in a horse immune to diphtheria-toxin. The estimations were made on two occasions, one before the commencement of immunisation and the other three months later, when the serum had reached a potency of 500 units. He found no essential increase of the total proteid contrary to what had previously been noted by Butjagin (1902) and Szontagh and Wellman (1898). The total globulin was, however, markedly increased at the expense of the albumin, but contrary to expectation the rise affected solely the euglobulin fraction which contained none of the antitoxin. His figures were:

Before immunisation,

Eugl. : Pseudogl. : Alb. :: 12·74 : 37·04 : 50·21.

After immunisation,

Eugl. : Pseudogl. : Alb. :: 26·21 : 36·74 : 37·05.

This remarkable result we shall discuss later.

The question of globulin variations in other experimental infections has been approached by Langstein and Mayer (1904), who showed that in rabbits immunised against typhoid, pneumococcus, sheptococcus, dysentery, cholera, and swine erysipelas the serum-globulin rose while

the albumin diminished. It was determined also by Mayer (1905) that the serum of dogs infected with *Trypanosoma* showed an increase of globulin and a diminution of albumin, although the total proteid was not markedly affected.

The objections which several authors have recently brought forward against the view that globulin-change is a necessary concomitant of the elaboration of antibodies will be discussed in the course of this paper in the light of our own observations.

#### *Description of Technique and Mode of Investigation.*

Though the main object of the present investigation was to ascertain by examination at frequent intervals whether any definite relationship could be traced during immunisation between the rise in potency and the globulin variations, it appeared desirable also to determine how far Pick's statements as to the antitoxin-contents of the various globulin fractions held good in the horse and goat.

For the first two series of investigations a horse (Plug) and a goat (*Mephistopheles*) were employed. The former was treated by the usual method which obtains at this institute, and previous to the commencement of immunisation estimations were made of the normal globulin and normal antitoxin present in the blood serum.

Half-saturation with ammonium sulphate was always employed to separate the total globulin from the albumin. The precipitate was redissolved in water and again treated with ammonium sulphate. After filtration the precipitate was again dissolved and coagulated by heat. The coagulated globulin was collected on a weighed filter paper, thoroughly washed with hot water and then dried in vacuo over sulphuric acid for several days. After a final dessication in an air-bath at 80° it was weighed.

The filtrates containing the albumin were also coagulated and weighed in a similar manner. In estimating the total proteid a fixed quantity of serum, 10 c.c., was diluted by addition of 190 c.c. of distilled water, and carefully acidified with acetic acid. It was then coagulated by heat and the precipitate dried and weighed. Where complete estimations of the euglobulin and pseudoglobulin were made, as well as of the total globulin and total proteid, three quantities of serum (each 10 c.c.) were used. With one quantity the total coagulable proteids were estimated, with another the total globulin and total albumin, and with the third quantity the euglobulin was obtained by third-saturation

with ammonium sulphate, the filtrates being employed for the estimation of the combined pseudoglobulin and albumin. The difference between this last and the albumin previously estimated, gave the amount of pseudoglobulin.

The potency of the serum was estimated in Ehrlich-Behring units by the usual methods practised in this Institute.

### *Immunisation of Horse (Plug).*

Immunisation was commenced on 17 Nov. 1905, with an inoculation of .01 c.c. toxin. Inoculations were made thereafter every third or fourth day, provided all local swelling had disappeared, each successive dose being double the preceding one.

In the following table (p. 72) are indicated the toxin-doses and the potency of the serum on the different dates.

According to the practice at this Institute the horses are bled nine days after they have received the final dose of 1000 c.c. toxin. The potency of the serum when tested at this time, *i.e.* after the first bleeding varies as is well known in different horses quite apart from the methods of immunisation employed. In our experience here the great majority of the horses attain a potency of 600 units and over, at the first bleeding, but there is always a small percentage of horses which do not reach 300 units. These latter are of course discarded so far as their employment for the production of diphtheria-antitoxin is concerned.

From the following table it will be seen that unfortunately for the object of our experiment, this horse proved unsuitable for the production of high grade antitoxin. Our hopes that the serum might attain a high antitoxin-content so that a suitable comparison might be made between this and the globulin-content were disappointed.

From the purely theoretical point of view, however, the facts brought by the immunisation of this refractory horse, present numerous points of interest and enable one to institute some comparison between the reaction to diphtheria-toxin of such horses and the more responsive animals.

On the 10th day after the first dose of 1000 c.c. toxin (2nd Feb. '06), (see Table I. and Chart I.), the serum had an antitoxin-content of only 180—200 units per c.c. One litre of blood was drawn and a further dose of 1000 c.c. given on 5th Feb. On the 9th day thereafter (14 Feb.) the potency had risen slightly to 240—250 units. On the 19th Feb., 22nd Feb., and 26th Feb. further doses of 500 c.c., 700 c.c., and 1000 c.c.

respectively, were administered with little appreciable effect on the potency.

TABLE I.

Date	Toxin-dose	Potency (in units)
Nov. 10 '05	—	$\frac{1}{20}$ — $\frac{1}{10}$ per c.c.
17	·01	—
20	·02	$\frac{1}{10}$ — $\frac{1}{4}$
23	·04	$< \frac{1}{2}$
27	·1	1—2
30	·2	2—4
Dec. 4	·5	2—4
7	1·0	$< 6$
11	2	4—6
15	4	—
19	8	8—12
22	15	8—12
26	30	12—16
29	60	12—20
Jan. 1 '06	120	$< 20$
4	250	20—28
8	500	40—50
12	500 (new tox.)	60—80
16	800	80—100
19	—	110
23	1000	110—120
26	—	140—160
31	—	160—180
Feb. 2	—	180—200
5	1000	—
9	—	$< 200$
14	—	240—250
19	500	—
22	700	—
26	1000	$< 240$
Mar. 7	—	250—300
12	1000 intraven.	—
21	—	$< 200$

Thus on 7th Mar. the serum had only between 250 and 300 units per c.c. A final attempt to raise the potency was made on 12th Mar. when 1000 c.c. toxin were injected intravenously by the jugular vein. The animal suffered no bad effects from the injection, but after a lapse of 10 days the potency instead of being raised had actually fallen below 200 units per c.c. In fact this latter experiment is in complete accord with a previous one made by Dr Dean and which goes to show that the intravenous injection of diphtheria-toxin has little or no effect in raising the potency in a horse whose antitoxin-content is falling.





TABLE II.

Date	Total proteid	Total globulin	Globulin percentage	Albumin
Oct. 30 '05	·8532	·7191	84	·1341
Nov. 20	·8816	·7608	87	—
23	·8330	·6618	79	—
27	·8702	·7529	86	—
30	·8034	·7400	92	—
Dec. 4	·8220	·7182	87	—
7	·8987	·6247	69	—
11	·8201	·5945	66	—
15	·8304	·5710	68	—
19	·8006	·5648	70	—
22	·8334	·6278	75	—
26	1·0622	·6532	61	—
29	·9382	·6582	70	—
Jan. 1 '06	·9273	·7213	77	·2060
4	·9372	·7064	75	·1696
8	·8906	·6074	68	—
12	·7460	·6653	89	—
16	·9294	·7390	79	—
23	·9711	·7727	79	·1478
Feb. 2	1·0272	·7357	71	·2370
14	·9561	·7204	75	—
Mar. 7	·9083	·6811	75	·1804
21	·8468	·5890	69	·1728

Horse (Plug).

In the above table (Table II.) are recorded estimations of the total coagulable proteid, and total globulin with a few albumin estimations. In all cases the amounts given are those derived from 10 c.c. serum. Starting with an initial value of ·8532 gm. on Oct. 30 before immunisation, the total proteid exhibits very slight fluctuations throughout the period ending Dec. 22 the average value being ·8406 gm. During the next period ending Jan. 23 the total proteid showed a slight though quite appreciable rise, the average for the period being ·9252 gm. From Jan. 23 onwards this higher level was maintained.

Regarding the globulin variations it will be seen in the first place that the initial value was much higher than that recorded by Hammarsten for the normal horse. In fact the value ·7191 gm. is nearly double the normal value. Thus three normal horses described by Hiss and Atkinson had initial values of ·3085 gm., ·4342 gm., ·3988 gm. in 10 c.c. serum while the horse "Wright," presently to be described, had an initial globulin value of ·4365 gm.

The fluctuations in the percentage globulin-content of the total proteid will be more readily followed from Chart I.

No progressive rise in the globulin-content occurred during the process of immunisation. While the total proteid remained stationary during the period Nov. 17 to Dec. 22 the globulin-content maintained its initial level only for a short time. It then fell persistently attaining its lowest level on Dec. 26.

During the second period when the total proteid was at a higher level, the globulin-content slowly rose to its initial value beyond which it never mounted. The values recorded in the period Jan. 23 to Mar. 21, show that a fall to a lower level had again occurred.

The rise in the total proteid from Dec. 22 onwards was due not to an increase of the globulin but of the albumin fraction.

The important question, whether our failure to obtain a high-grade antitoxic serum in this horse, is in any way related to the peculiar behaviour of the globulin, will be discussed later.

#### *Immunisation of Goat (Mephistopheles).*

In the immunisation of the goat, almost exactly the same procedure was adopted as in the case of the horse except that the initial dose of toxin was only '001 c.c.

The inoculations were made not intramuscularly but subcutaneously in the dorsal region over the erector spinae mass. Before immunisation the normal serum was tested for the presence of normal antitoxin and quantitative estimations of the proteids made. Further in view of the possibility of globulin-variations due to lowered condition etc. the animal was carefully weighed every week. The annexed table (Table III.) shows the scheme of immunisation and the potency of the serum on the different dates.

It was found that the toxin doses were well tolerated up to the middle of February when the animal began to show signs of weakness. After the dose of 100 c.c. the goat developed slight paresis in the hind limbs but appetite remained good and a further injection of 150 c.c. was made on the 19th February. The paresis however became worse and latterly affected the fore limbs so that the animal had to lie on its side. Though thus prostrated appetite remained good but death took place suddenly on 3rd March probably from heart failure.

At the post mortem the stomach was enormously dilated with food-stuffs. Apart from haemorrhagic oedema of the lower pulmonary lobes there were no gross changes in the organs. The serum obtained post mortem had between 20 and 30 units per c.c.

TABLE III.

Date	Toxin-dose	Weight	Potency (in units)
Nov. 6 '05	—	—	$< \frac{1}{8}$
9	—	—	$< \frac{1}{20}$
20	—	—	$\frac{1}{40}$
Dec. 1	·001 c.c.	90 lbs.	—
5	·002	—	—
9	·004	—	—
13	·01	89	—
18	·02	—	—
23	·04	—	$< \frac{1}{3}$
27	·1	80	—
30	·2	—	—
Jan. 3 '06	·5	88	—
8	1	—	—
12	2	87	—
16	3	—	4
19	6	80	—
23	12	—	5—10
29	25	—	—
Feb. 3	50	—	$< 5$
7	50 (new tox.)	—	—
12	100	—	5—10
19	150	—	15—20
Mar. 3	Death	—	20—30

From the potency curve (see Chart II.) it will be seen that it required six weeks' immunisation to raise the antitoxin-content to one unit per c.c. Thereafter progress was more rapid, but for a period of three weeks, Jan. 23 to Feb. 12, the potency appears to have remained more or less stationary with an interval of depression.

After the 12th Feb. the antitoxin-content rose markedly and continued to do so up to the fatal conclusion in spite of the diphtheritic paralysis. Records of immunisation of goats against diphtheria-toxin are not numerous and it does not appear that these animals are capable of producing serum of high potency.

In one goat previously immunised at this Institute a potency of 40 units was reached. The goat serum with which Pick worked had a very low antitoxic value inasmuch as ·1 c.c. was required to neutralize 10 lethal doses of a toxin whose M.L.D. was only about ·01 c.c. (The toxin employed here in the immunisation of the horses and goat had a M.L.D. of ·002 c.c.)

In Table IV. are recorded the values of the total proteid, total globulin and albumin, during the immunisation of the goat. The figures for the normal serum of Nov. 6 show, as in the horse, a marked

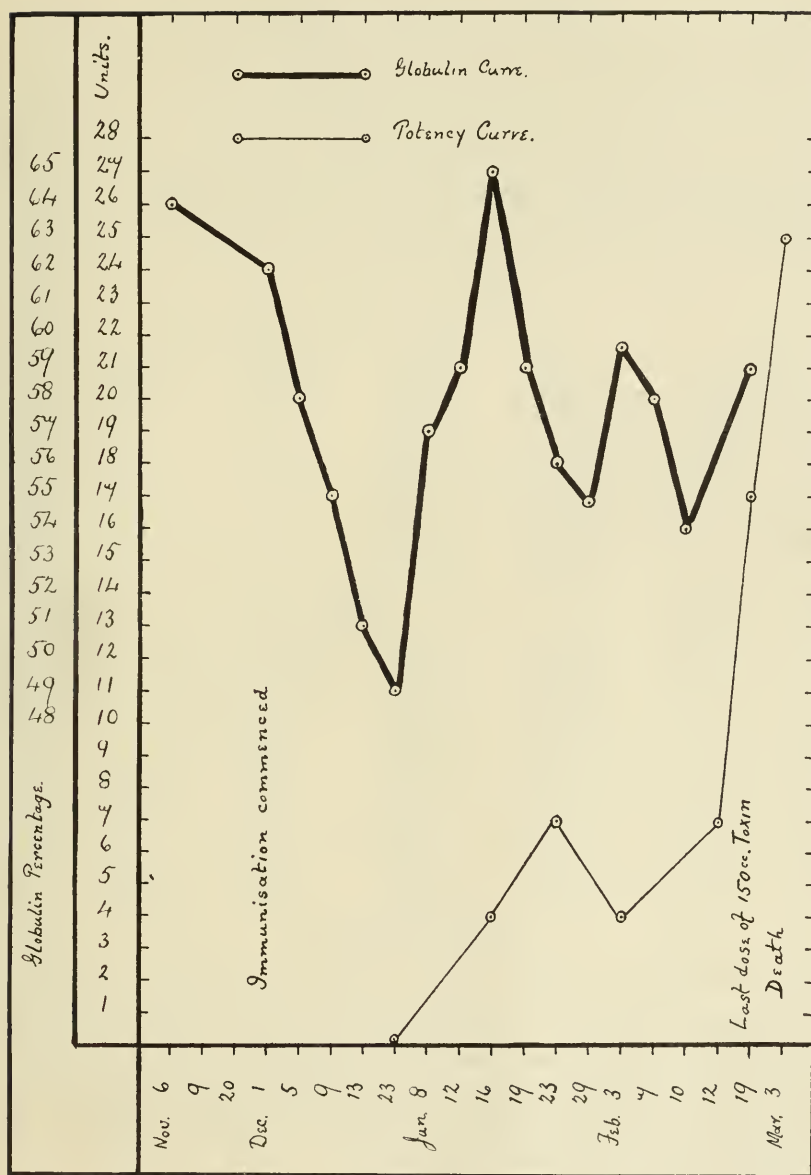


CHART II.

Chart showing the course of antitoxin-development and the variations in the percentage globulin-content of the total proteid, during the immunisation of goat (*Mephistopheles*).

preponderance of the globulin—over the albumin—fraction. The total proteid shows a pronounced and fairly progressive rise from .5974 gm. on Dec. 1 to .8555 gm. on Jan. 29. This increase which was equivalent to one of 43 % on the initial value greatly exceeded in amount the corresponding increase in the horse "Plug," but when we consider the tables we find that the very slight increase of total globulin during immunisation, is quite insufficient to account for the greatly increased total proteid. Indeed, as will be apparent from Chart II, the percentage globulin-content of the total proteid fell markedly during the first three

TABLE IV.

Date	Total proteid	Total globulin	Globulin percentage	Albumin
Nov. 6 '05	.5867	.3775	64	.2092
Dec. 1	.5974	.3739	62	—
5	.6668	.3868	58	—
9	.6908	.3820	55	.2261
13	.6161	.3156	51	—
18	.6388	—	—	—
23	.6428	.3204	—	—
27	—	—	—	—
30	—	—	—	—
Jan. 3 '06	—	—	—	—
8	.7430	.4252	57	—
12	.7410	.4399	59	—
16	.7150	.4644	65	—
19	.7608	.4548	59	—
23	.8128	.4554	56	—
29	.8555	.4702	54.9	—
Feb. 3	.7615	.4547	59.7	.2873
7	.7669	.4476	58	—
10	.7581	.4098	54	—
19	.6852	.4047	59	.2597

Goat (*Mephistopheles*).

weeks. It then rose gradually till it attained a value slightly above the initial figure of 64 %. The rise, however, was only temporary and was succeeded by a second fall which lasted with slight fluctuations during the period of maximum potency. It must be said, therefore, that the great increase in the total proteid was due in far greater measure to the albumin than to the globulin fraction

Such a result, in view of the fact that the globulin is the antitoxin carrier, seems rather surprising and will demand our consideration later.



*Immunisation of Horse (Wright).*

As the horse "Plug" proved unsatisfactory for the production of high grade antitoxin and consequently did not permit of our drawing any very definite conclusions regarding the relationship of the serum-globulins to the potency, it was decided to immunise another horse.

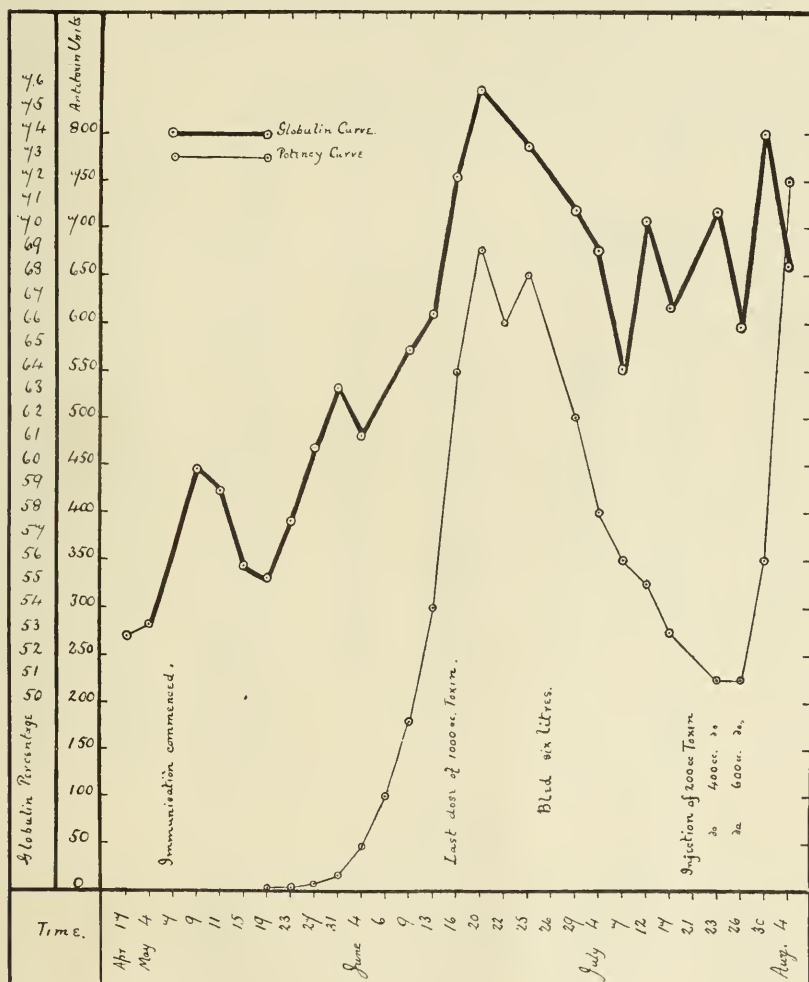


CHART III.

Chart showing the course of antitoxin-development and the variations in the percentage globulin-content of the total proteid during the immunisation of horse (Wright).

The procedure adopted was in all respects similar to that in the case of "Plug" except that the inoculations were made, where possible every second day. No delay was experienced until doses of 200, 400, and 600 c.c. were administered when the inoculations were postponed for a day or two to allow local swelling to subside.

In the following table (Table V.) are recorded the toxin-doses and potency of the serum during the course of immunisation.

It will be observed that "Wright" reacted in a much more characteristic way to diphtheria-toxin than "Plug," and the potency curve (see Chart III.) may be considered a fairly typical one.

TABLE V.

Date	Toxin	Potency (in units)
April 17 '06	—	$< \frac{1}{4}$
May 7	·01	—
9	·02	—
11	·04	$< \frac{1}{3}$
13	·1	—
15	·2	—
17	·5	—
19	1	2—4
21	2	—
23	4	2—4
25	8	—
27	15	8
29	30	—
31	50	16
June 2	100	—
4	200	$> 40$
6	400	100
9	600	180
13	—	300
16	1000	550
20	—	675
22	—	600
25	—	650
26	Bled 6 litres	—
29	—	500
July 4	—	400
7	—	350
12	—	325
17	—	250—300
21	200	—
23	400	225
26	600	200—250
30	—	$> 300$
Aug. 4	Bled 4 litres	750

After the first bleeding, the potency gradually fell to 300 units and under, but a second series of inoculations caused it to mount upwards again to a level higher than that attained at the end of the first series. Such a phenomenon is not unfrequent.

In Table VI. are recorded the values of the total proteid, total globulin and albumin during immunisation. A series of euglobulin and pseudoglobulin estimation are also entered.

TABLE VI.

Date	Total proteid	Total globulin	Albumin	Euglobulin	Pseudo- globulin	Globulin percentage	Albumin percentage
Apr. 17 '06	·8266	·4365	—	—	—	52·8	—
May 4	·7954	·4241	—	—	—	53·3	—
9	·8440	·5050	·2734	·1600	·3333	59·8	32·3
11	·7964	·4692	—	—	—	58·9	—
15	·8289	·4622	·2636	·0952	·3544	55·7	31·8
19	·7278	·4021	·2826	—	—	55·2	38·8
23	·7884	·4546	·2545	—	—	57·6	32·2
27	·8226	·4998	·3027	·0916	·3893	60·7	36·7
31	·8043	·5094	·3005	·1495	·3131	63·3	37·3
June 4	·8065	·4941	·3046	·1358	·3072	61·2	37·7
9	·8346	·5419	·2456	—	—	64·9	29·4
13	·9452	·6278	·1930	·2178	·4295	66·4	20·4
16	1·0234	·7379	·2363	·1146	·6040	72·1	23·0
20	1·0600	·8046	·2004	·2934	·4080	75·9	18·9
25	·9387	·6893	·2043	·2795	—	73·4	21·7
29	·8519	·6026	·1916	·1320	·4286	70·7	22·5
July 4	·8190	·5655	·2151	—	—	69·0	26·2
7	·7963	·5102	·2333	—	—	64·0	29·3
12	·8225	·5785	·2596	—	—	70·3	31·5
17	·7853	·5238	·2874	—	—	66·7	36·6
23	·8398	·5940	—	—	—	70·7	—
26	·9342	·6159	—	—	—	65·9	—
30	·9429	·6986	—	—	—	74·0	—
Aug. 4	·9276	·6343	—	—	—	68·3	—

Horse (Wright).

During the first month the total proteid remains fairly constant with slight fluctuations, but a rapid rise takes place between the 9th and 20th June, a period corresponding to the maximum antitoxin development. This rise is succeeded by an appreciable fall during the resting period following the bleeding. A second rise, however, makes its appearance after the second series of inoculations. Roughly the total proteid rose from 8% before immunisation to 10% at the period of maximum antitoxin development.

Apart from slight fluctuations, the absolute amount of globulin rises very slowly during the first three weeks so that the percentage globulin-content of the total proteid also rises (see Chart III.). The increase is much more rapid in the second month when the potency is rising the figure for 20 June being almost double the initial figure before immunisation. At the period of maximum globulin-content the albumin percentage is reduced to 18.9 %.

We have, therefore, to record in this horse a very marked rise in the globulin-content coincident with the development of a high potency and with a reduction of the albumin fraction. Regarding the euglobulin and pseudoglobulin estimations the results are somewhat indefinite but would at least indicate that in the globulin increase, both fractions are implicated and the euglobulin more so than the pseudoglobulin. The result is in more or less agreement with that recorded by Joachim and to which we have already referred.

After the bleeding and during the resting stage the total proteid and total globulin fall while the albumin remains more or less stationary or even tends to rise. When the inoculations were renewed, the total proteid and total globulin again rose but more slowly and even at the period when the potency had reached its second maximum the total globulin had not quite reached a value equal to its previous maximum at the end of the first series of inoculations. The globulin values, however, never attain the low limits prevailing before the commencement of immunisation and during the earlier part of the process.

The question of the influence of inanition or repeated bleedings, on the constitution of the blood plasma has undoubtedly to be considered in all experimental work designed to bring out a causal connexion between rise in potency and rise in the globulin fraction of the blood serum. In this connexion we may cite the work of Githens (1904) who found that in hungering dogs a rise in the globulin fraction occurred especially affecting the fibrinoglobulin and euglobulin, while after repeated bleedings the albumin-content rose markedly.

This peculiar relationship of the albumin and globulin fractions inclined the author to believe that the albumin behaves as if it came nearest to the "Nahrungseiweiss" and suggested the possibility that the globulins under these conditions might be derived from the albumins either inside or outside the circulating blood.

Glässner (1905) investigated the changes in the blood plasma before and after the immunisation of rabbits with bacterial emulsions, bacterial

toxins and proteid bodies as horse or ox sera. He came to the conclusion that a rise in the globulin fraction was only marked in those animals which had lost weight during immunisation, and that consequently a rise in the globulin fraction could not be considered an essential concomitant of the formation of antibodies but only as a secondary symptom depending on inanition. Provided immunisation is cautiously proceeded with and the animals not allowed to get into an enfeebled condition, a rise in globulin need not necessarily follow. Glässner thinks his views are supported by the results obtained by Githens already quoted but as has also been pointed out recently by Moll (1906) the globulin values obtained by Githens after inanition are not to be compared to the high values obtained by immunisation.

In Moll's own experiments on fasting dogs and rabbits no marked rise in the globulin fraction was noted. In two cases the globulin even fell. By immunising with horse serum however a great rise in globulin was observed and was accompanied by the development of marked precipitin formation. He does not believe that the rise in globulin is an inanition effect though it is possible that pure inanition may influence this phenomenon during immunisation.

Before commencing our work we had in view the possibility of inanition or loss of weight as possible disturbing factors. Unfortunately the horses were not weighed during immunisation but it is the general experience in this Institute that, so far from losing weight the horses appear actually to put on flesh. They receive as much nourishment as they will take and enjoy regular exercise. It is questionable, however, how far we are to consider this apparent well-being as a criterion of an undisturbed metabolism. The occasional occurrence of waxy disease, hepatic haemorrhage etc. causing sudden death in apparently healthy horses undergoing immunisation for long periods, suggests that metabolism may be profoundly altered without much visible sign being afforded of its effects.

*Determinations of the antitoxin-contents of the proteid fractions  
in the horse and goat.*

We have already referred to Pick's work showing that in the horse the pseudoglobulin is the antitoxin carrier, while in the goat the euglobulin performs that function. As no confirmatory evidence on this important question is yet available, and as the possibility was always present that the sera of different horses might comport themselves



differently in regard to the antitoxin-contents of their respective fractions, it seemed desirable to test the potency of a number of proteid solutions isolated from horse and goat serum at different stages in immunisation.

The separations were all performed by the ammonium sulphate method and the various precipitates were dissolved in distilled water. Where the final fluid exceeded in bulk that of the original serum quantity taken, the necessary allowance was made in testing the potency.

For each test three or four guinea-pigs of standard weight were employed.

### *Experiments on Horse serum.*

#### *Exp. I. Total globulin.*

Serum employed: Plug's serum of March 7, 1906 containing 250—300 units per c.c.

Mode of separation: A single precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to half saturation.

Dissolved precipitate tested for 100, 200 and 250 units in a series of three pigs.

Result: Pig No. (3) died on 4th day while others lived.

Remarks: The globulin contained practically all the antitoxin.

#### *Exp. II. Total albumin.*

Mode of separation: Filtrate from Exp. I.

Tested for 10, 20 and 40 units in three pigs.

Result: All three pigs died on 2nd day.

Remarks: Apparently the albumin contains none of the antitoxin.

#### *Exp. III. Euglobulin.*

Serum employed: Plug's serum of March 21, 1906 containing 200 units per c.c.

Mode of separation: A single precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to one-third saturation.

Tested for 10, 20 and 40 units in three pigs.

Result: All three pigs lived.

Remarks: No conclusion can be drawn as a certain amount of the pseudoglobulin may have been mechanically carried down in the first precipitate.

#### *Exp. IV. Pseudoglobulin-albumin.*

Mode of separation: Filtrate from Exp. III.

Tested for 50, 100 and 150 units.

Result: Pig No. (1) lived, pig No. (2) died on 4th day, and pig No. (3) died on 2nd day.

Remarks: The combined pseudoglobulin and albumin contained more than half the antitoxin after a single separation from euglobulin by one-third saturation.

*Exp. V. Euglobulin.*

Same serum as in Exp. III.

Mode of separation : Complete separation of euglobulin by repeated precipitation with  $(\text{NH}_4)_2\text{SO}_4$ .

Tested for 10, 20 and 40 units.

Result : Pig No. (3) died on 2nd day while the others lived.

Remarks : The euglobulin fraction even after complete separation from the pseudoglobulin and albumin contained 20 units per c.c.

*Exp. VI. Pseudoglobulin-albumin.*

Mode of separation : Filtrate from Exp. V.

Tested for 50, 100 and 150 units.

Result : Pig No. (3) died on 3rd day while others lived.

Remarks : The pseudoglobulin-albumin therefore contained three-fourths of the antitoxin.

*Exp. VII. Euglobulin.*

Serum employed : Serum of "Togo" containing 600 units per c.c.

Mode of separation : Single precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to one-third saturation.

Tested for 25, 50 and 100 units.

Result : All three pigs lived.

Remarks : The remarks on Exp. III. apply here also.

*Exp. VIII. Euglobulin.*

Same serum as in Exp. VII.

Mode of separation : Complete separation from pseudoglobulin by repeated precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to one-third saturation.

Tested for 25, 50 and 100 units.

Result : All three pigs died on 2nd day.

Remarks : The euglobulin apparently contains none of the antitoxin.

*Exp. IX. Pseudoglobulin-albumin.*

Mode of separation : Combined filtrates from Exp. VIII.

Tested for 400, 500 and 600 units.

Result : Pig No. (1) lived while pig (2) and (3) died on 2nd day.

Remarks : The pseudoglobulin-albumin certainly contained over two-thirds of the antitoxin but some loss of antitoxin has apparently occurred in the preparation of the different fractions.

*Experiments on Goat serum.**Exp. I. Total globulin.*

Serum employed : Mephistopheles' serum of 19 Feb. 1906 containing 15—20 units per c.c.

Mode of separation : Complete separation from albumin by repeated precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to half saturation.

Tested for 5, 10 and 15 units.

Result : Pig No. (3) died on 3rd day while the others lived.

Remarks : The globulin appears to contain all the antitoxin.

*Exp. II. Euglobulin.*

Serum employed: Meph. serum of Feb. 7, 1906 containing about 5 units per c.c.

Mode of separation: Single precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to one-third saturation. Tested for 3, 4 and 5 units.

Result: Pig No. (3) died on 3rd day while the others lived.

Remarks: The euglobulin appears to contain all the antitoxin but the experiment is not conclusive, as some of the pseudoglobulin may have been carried down in the first precipitation.

*Exp. III. Pseudoglobulin-albumin.*

Mode of separation: Filtrate from Exp. II.

Tested for 2, 3 and 4 units.

Result: All three pigs died on 2nd day.

Remarks: Evidently a single precipitation with  $(\text{NH}_4)_2\text{SO}_4$  to one-third saturation removed all the antitoxin, leaving none in the pseudoglobulin-albumin filtrate.

*Exp. IV. Euglobulin.*

Serum employed: Meph. serum of Jan. 29, 1906 containing about 5 units per c.c.

Mode of separation: Complete separation from pseudoglobulin-albumin by repeated one-third saturation with  $(\text{NH}_4)_2\text{SO}_4$ .

Tested for 2, 3, 4 and 5 units.

Result: All pigs died on 2nd day.

Remarks: It would appear that on this occasion none of the antitoxin was contained in the euglobulin fraction.

*Exp. V. Euglobulin.*

Serum employed: Meph. serum of 19 Feb. 1906, containing 15—20 units per c.c. (same serum as in Exp. I.).

Mode of separation: Complete separation from pseudoglobulin-albumin by repeated one-third saturation with  $(\text{NH}_4)_2\text{SO}_4$ .

Tested for 5, 10 and 15 units.

Result: All three pigs died on 1st day from toxæmia.

Remarks: Taken in conjunction with Exp. I. it would seem that on this occasion all the antitoxin must have been contained in the pseudoglobulin fraction.

*General Summary of Experimental results.*

There appears to be no doubt that the greater part, if not the whole of the antitoxin of horse serum is contained in the pseudoglobulin fraction. In the horse "Togo" which was yielding serum of high potency not more than 2—3% of the antitoxin could have been present in the euglobulin when completely separated from the pseudoglobulin.

In the horse "Plug," however, the euglobulin when completely separated from the pseudoglobulin still contained fully 10% of the antitoxin. It will be remembered that from Feb. 14, 1906 onwards

little change took place in the potency in spite of repeated stimulation. The serum of Mar. 21 (see Exp. V.) was drawn at a time when the potency was evidently falling or at least stationary so that the conclusion is forced upon us that in the serum of a refractory horse which responds sluggishly or not at all to stimulation there may be no such sharp delimitation of antitoxin to the pseudoglobulin as in the serum horses more susceptible to stimulation.

In order to obtain reliable data on this point, however, it would be necessary to make a larger series of experiments on the above lines, at different stages in the immunisation of refractory horses.

In the case of the goat I am unable to confirm Pick's statement that the antitoxin is invariably linked on to the euglobulin fraction. The analysis of sera on 29th Jan. and 19th Feb. showed that the euglobulin contained none of the antitoxin, while on the intervening date, 7th Feb., the euglobulin (though here incompletely separated) contained all the antitoxin. It is interesting to observe that between the 23rd Jan. and 12th Feb. the potency remained stationary with an intervening depression so that again we have the possibility suggested to us that during a refractory period which corresponds in all probability to an abnormal metabolic activity, the distribution of the antitoxin-molecules may be altered.

The fact too that in the globulin-increase during immunisation the euglobulin appears to take a larger share than the pseudoglobulin which acts as the antitoxin-carrier seems to show that some compensatory mechanism is at work. In the horse whose reaction to stimulation is prompt and is evidenced by increased potency, we may presume that a more or less constant number of pseudoglobulin molecules has to combine with or hold together a very largely increased number of antitoxin molecules. In order to prevent any diversion of these pseudoglobulin molecules to simple functions of nutrition the euglobulin undergoes what one may call a "compensatory hypertrophy."

In the refractory animal, however, or during a period of stationary potency such a mechanism may not exist, or once established may readily be upset in which case a redistribution of the antitoxin molecules would not be an unexpected phenomenon. Hitherto no explanation has been afforded of the different reactions displayed by different horses to diphtheria-toxin. From the data furnished by the immunisation of "Plug" and "Wright" there would seem to some intimate relation between the amount of antitoxin developed and the quantity and quality of the globulins. In both cases (as also in the goat whose power

of elaborating antitoxin is very limited) the total proteid was increased, but in the first case the increase affected largely the albumin, and in the second case, the globulin solely. In the goat the albumin was mostly implicated in the increase of total proteid. Now both horses had practically the same amount of total proteid initially but in the case of "Plug" the globulin fraction preponderated enormously over the albumin fraction. It was only at the height of antitoxin-production that the globulin of "Wright" attained a value equal to that of "Plug" initially. Had the globulin fraction of "Plug's" serum increased in the same proportion as that of "Wright," quite apart from increase of potency, the total proteid would have consisted practically entirely of globulin.

But there is no doubt a physiological limit to the amount of globulin in serum and that limit was attained in "Plug," for some unexplained reason, before immunisation began. Hence the increase in the total proteid fell largely on the albumin fraction which however does not functionate as an antitoxin carrier. If in view of Hiss and Atkinson's work and the data supplied by "Wright" we are to consider increase of globulin as a necessary concomitant of high grade antitoxin-development then the failure of "Plug" to yield such high grade serum is not surprising.

We use the term "high grade serum" advisedly because there is no doubt that a certain degree of potency is attainable without any very marked disturbance in the constitution of the serum-proteids. In this connexion it seemed advisable to exclude any possible globulin variation that might occur from the injections of large quantities of bouillon alone, apart from the toxin they contain. To make certain on this point a horse "Lister" was inoculated with increasing quantities of alkaline bouillon according to the following scheme.

The bouillon employed was the same as that used in the production of diphtheria-toxin. It was kept in the incubator for ten days and finally filtered through a Berkefeld in order to approximate as closely as possible the conditions of the toxin-containing bouillon.

The initial dose which was inoculated intramuscularly was 50 c.c. Thereafter doses of 100, 200, 400, and 800 were given at intervals of two or three days.

After each injection a well marked pyrexia was evident but otherwise no untoward symptoms appeared.

Before the commencement of the inoculations estimations were made of the total proteid, total globulin and albumin.

In Table VII. are recorded the various proteid estimations throughout



the period of inoculation. It will be seen that no appreciable change took place in the amount of total proteid. The globulin fluctuations were also very slight as will also be evident from Chart IV. which shows the fluctuations in the percentage globulin-content of the total proteid.

TABLE VII.

Date	Total proteid	Total globulin	Globulin percentage	Albumin
July 31 '06	·7493	·4880	65·1	·2702
Aug. 8	·7898	·4654	58·9	—
13	·8063	·5246	65	·3201
16	·8478	·5006	59	·2074
20	·7590	·4639	61·1	·2070

Horse (Lister).

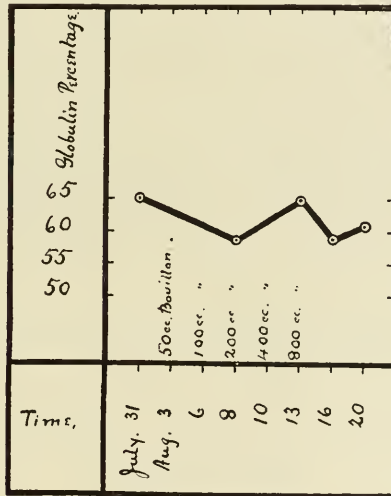


CHART IV.

Chart showing the variations in the globulin-content of the total proteid during the course of the Bouillon-injections (Lister).

We may therefore exclude the inoculation of large quantities of bouillon as a possible factor in the production of the marked globulin-increase which occurs during the immunisation of susceptible horses with diphtheria-toxin.

*Summary and Conclusions.*

1. During the immunisation of a horse which ultimately failed to yield high grade antitoxic serum, the globulin-content of the total proteid showed no tendency to increase. The slight rise in total proteid which occurred was due to an increase in the albumin fraction. It is probable that the failure of this horse to yield high grade antitoxin was in some way connected with the initial high globulin-content of the serum.

2. During the immunisation of a goat, the rise in total proteid affected mainly the albumin fraction and the globulin fraction in lesser degree.

3. During the immunisation of a horse which ultimately yielded high grade antitoxic serum, the percentage globulin-content of the total proteid, progressively increased. This increase affected the euglobulin fraction more than the pseudoglobulin fraction.

4. In the horse the pseudoglobulin contains the greater part if not all the antitoxin but it seems probable that this relationship holds good only when the antitoxin-content of the serum is steadily rising.

5. In the goat the antitoxin-content of the euglobulin and pseudoglobulin fractions may vary at different periods in the course of immunisation.

In conclusion I have to express my thanks to Dr Dean the Bacteriologist-in-charge for kindly help and suggestions in the course of this work.

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### ADDENDUM.

In the *Proceedings of the Society for Experimental Biology and Medicine*, vol. IV, No. 1, New York, Dec. 1, 1906, is contained the abstract of a paper by Gibson and Collins on the fractionation of agglutinins and antitoxin. These authors report:—"Precipitation of anti-diphtheria goat serum showed that about half the antitoxin remained in the pseudoglobulin; practically none was found in the euglobulin while the  $\frac{1}{3}$ rd saturated  $(\text{NH}_4)_2\text{SO}_4$  solution washings contained the balance." The results of their experiments, so far as they had gone, appeared to indicate the unreliability of Pick's differentiation of the antibodies by fractional precipitation of the globulins.

## NOTES ON THE LEUCOCYTE-REACTION DURING THE IMMUNISATION OF THE HORSE AND GOAT WITH DIPHTHERIA TOXIN.

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IN the foregoing paper details have been given of the immunisation with diphtheria toxin of three animals (two horses and one goat). In the case of the horse "Plug" and goat "Mephistopheles" the opportunity was taken of investigating the question of the leucocyte reaction with a view to determine whether any notable changes in the leucocytic formula took place as the result of the frequent injections of toxin and the increased elaboration of antitoxin. The few investigations which have been recorded on the blood-changes during diphtheria immunisation have unfortunately led to somewhat contradictory conclusions.

Nicolas and Courmont (1897) were the first to study this question in any detail. These authors recorded the variations in the total leucocytes during the immunisation of several horses. Immunisation was begun with iodised toxin and continued every second or third day with gradually increasing doses of pure toxin. No fixed rule was followed however. The final dose after about fifty injections was only 60 c.c. toxin. Consequently the intermediate doses of 5 c.c., 10 c.c., 15 c.c., 20 c.c., 30 c.c., 40 c.c., and 50 c.c., were many times repeated. Counts made at rather irregular intervals during the course of immunisation did not show any reaction in the direction of hyperleucocytosis. The average leucocyte count during immunisation varied only very slightly from that previous to the commencement of the inoculations. On two or three occasions, counts were made every two or three hours, after the injection, but here again no notable reaction was evident. Nicolas and Courmont concluded that no conspicuous leucocyte-reaction was to be

detected either at the beginning or at an advanced stage of the injection period and that therefore immunity could be developed without any essential leucocyte change. If hyperleucocytosis results at all it is merely a sign of grave intoxication and indicates that the toxin dose has been too strong.

The work of these authors was severely criticised by Besredka (1898) who laid stress on the necessity of paying attention principally to the behaviour of the polynuclear cells and not of the absolute leucocyte count. Further, while pointing out that Nicolas and Courmont had failed to give any account of the antitoxin yield of their horses, Besredka affirmed that he would not have been surprised at the absence of leucocyte-reaction in the absence of any great development of immunity. It seems doubtful how far this latter criticism of Besredka is justifiable.

This author immunised a goat with diphtheria toxin and showed that each dose of toxin was followed by a conspicuous leucocyte reaction in which the polynuclear cells were mainly implicated. During the later stages, however, there was some evidence that the polynuclear cells did not react so promptly and that the mononuclear cells participated more largely in the reaction.

It may be noted that immunisation was begun with heated toxin and followed up with the unheated fluid. The final dose, after about three months' immunisation, was only 1·5 c.c. of a toxin whose lethal dose for a guinea-pig of 500 gm. was  $\frac{1}{50}$  c.c. At the end of this period he was able to demonstrate that ·5 c.c. of the goat serum when mixed with 20 fatal doses of toxin (= ·20 c.c.) protected a guinea-pig of 500 gm. weight. No mention of his method of taking blood-samples from the goat is made by Besredka.

In reply to the latter's criticism Nicolas and Courmont (1898) maintained that their horses had undoubtedly developed antitoxin as a result of the injections. Further, they refer to the temporary paraplegia of Besredka's goat as being due to incautious inoculation. Under such conditions, they assert, a certain degree of hyperleucocytosis would not be an unexpected phenomenon.

A second contribution to the subject was made by Nicolas, Courmont and Prat (1900). Three animals were immunised, a horse, a goat, and a donkey. The polynuclear cells were estimated in addition to the absolute counts. Each animal received increasing doses of toxin up to 17 c.c., which was the final dose. In the goat the initial dose was  $\frac{1}{500}$  c.c. of iodised toxin. The counts were made every two or three days, but no hyperleucocytosis was ever demonstrable. Indeed, it



appeared that a hyperleucocytosis associated with a hypopolynucleosis was most in evidence during the immunisation. In summing up their results these authors adhere to their previous statements that immunisation is possible without any essential leucocyte reaction. Butjagin (1902) without giving numerical details, agrees with Nicolas and Courmont, that the elaboration of antitoxin need not be associated with any notable leucocyte reaction.

*The leucocyte reaction in the goat.*

In the case of "Mephistopheles" (as also of "Plug") the samples were always taken from the jugular vein by cannula, as it was found that samples taken from the ear gave fallacious results. The skin of the goat's ear is exceedingly tough and elastic, so that after puncture of the vein the blood did not flow freely but tended to gather in the subcutaneous tissue, forming a lump which was slowly absorbed. In Table I. are recorded, in relation to the toxin doses the total leucocyte counts and the percentages of the various leucocyte forms during the immunisation of the goat.

From Dec. 1 to Feb. 7 the counts were made every four days, corresponding with the toxin-injections. After the large doses inoculated on Feb. 7 and 12, counts were made every day, in order to determine more accurately the course of the leucocytes in response to massive doses. Also, on several occasions prior to the commencement of immunisation, the leucocytic formula was determined, the percentage of the non-granular cells being about 10% higher than that of the polynuclear cells. On the first day of the injections the relation of the non-granular cells to the polynuclear cells was as 55.2 : 43.6.

Let us consider the period from Dec. 1 to Jan. 16. It will be seen from the table that the effect of one injection had generally disappeared before the next came on, with notable exceptions on Dec. 9, Dec. 30 and Jan. 16. On these latter occasions a marked relative polynuclear leucocytosis still remained after the preceding injection. Also on Dec. 30 and Jan. 16 the absolute leucocyte counts were comparatively high.

In the period from Jan. 19 to Feb. 7 the polynuclear cells predominated markedly on the fourth day after each injection. The daily counts after the injection of 50 c.c. toxin on Feb. 7 and of 100 c.c. toxin on Feb. 12, show clearly that the slight resultant hyperleucocytosis is mainly due to an increase in the polynuclear cells while the mononuclears show only slight fluctuations.

TABLE I. Goat (Mephistophiles).

Date	Toxin	Total leucocytes	Small lymphs.	Large lymphs.	L. monos. and trans.	Non-gran. percentage	Polymorphos.	Eosinophils	Mast-cells
Dec. 1	·001	9,350	51·6	2	1·6	55·2	43·6	·8	·4
5	·002	9,650	48	6·1	3·5	57·6	40	1·7	·03
9	·004	9,200	40·6	2·8	1·8	45·2	53·2	1	·6
13	·01	8,300	38	9·5	6·2	53·7	43·7	1·7	·7
18	·02	6,650	51·8	4·4	1	57·2	41·2	1·2	·4
23	·04	8,350	71·1	1·1	·9	73·1	24·3	2·4	0
27	·1	7,900	48·8	2·9	·1	51·8	45·6	1·8	·6
30	·2	11,000	25·3	3·4	·5	29·2	65	4·8	·9
Jan. 3	·5	7,700	49·8	4·4	1·7	55·9	42·3	·8	·9
8	1	6,850	55·3	8·6	·1	64	33·3	2·6	·1
12	2	6,400	41·9	7·9	2·6	52·4	44·2	2·6	·7
16	3	10,500	23·6	4·6	2·8	31	67·9	·8	·2
19	6	9,500	41·5	5·2	·2	46·9	52	·9	0
23	12	8,300	38·8	1·2	0	40	56·6	2·6	·7
29	25	5,800	39·9	7	1·9	48·8	47·5	3·3	·2
Feb. 3	50	8,000	38·2	1·1	·4	39·7	58·4	1·2	·5
7	50	7,350	45·1	1·8	·8	47·7	50·8	1·1	·4
8	—	7,750	32·3	5·3	1	38·6	60·7	·2	·4
9	—	11,400	36·3	1·5	·9	38·7	60	·6	·6
10	—	7,500	29·3	1·1	1·4	31·8	67·4	·3	·3
12	100	6,400	39	3·4	·9	43·3	55	1·2	·2
13	—	10,600	30·4	·9	·6	31·9	67·6	·3	0
14	—	12,000	30·8	1·4	1·9	34·1	65	·7	0
15	—	8,400	38·4	2·1	·9	41·4	57·3	·9	·3
16	—	9,000	51·7	2·6	0	54·3	43·8	·9	1
19	150	11,400	30·5	·3	0	30·8	68·6	·5	0
20	—	16,000	14·9	·4	0	15·3	84·6	0	0

The absolute values of the non-granular and polynuclear cells after Feb. 7 and 12 are recorded below.

Date	Non-granular	Polynuclear	
Feb. 7	3431	3650	Inoculation of 50 c.c.
8	2926	4620	
9	4332	6840	
10	2325	5025	
11	—	—	
12	2752	3520	Inoculation of 100 c.c.
13	3286	4982	
14	4080	7800	
15	3444	4788	
16	4860	3870	
17	—	—	Inoculation of 150 c.c.
18	—	—	
19	3420	7752	
20	2400	13440	

On the day following the inoculation of 150 c.c. a very marked hyperleucocytosis was present and the polynuclear percentage had mounted as high as 84.6.

Regarding the behaviour of the eosinophile cells and mast-cells no very definite conclusions can be drawn owing to the small numbers of these cells present in the blood of the goat. It would seem, however, that after the injections on Feb. 7, 12, and 19, the eosinophile percentage was considerably reduced.

In Table II. are recorded the leucocyte estimations made during the immunisation of "Plug." Before the commencement of the injections the normal leucocytic formula was ascertained. In marked contrast to the goat, the polynuclear percentage slightly exceeds the non-granular percentage. The leucocytic formula of the horse is also remarkable on account of the high percentage of eosinophile cells.

Let us consider the period from Nov. 17 to Jan. 8.

Although a slight rise in the total leucocyte count was evident on the third day after each of the earlier injections, the percentages of the non-granular and polymorphonuclear cells had invariably returned to their normal values.

On Dec. 4, 15, and 19, however, a marked polynucleosis remained. Also on the 19th the total count remained at the high value of 18,000. It is interesting that the further injection of 8 c.c. toxin on this date did not produce any cumulative effect on the leucocyte reaction as, on the following day, the total count had fallen and the relative polynucleosis had diminished.

Date	Toxin	Total leucocytes	Small lymphs.	Large lymphs.	L. monos. and trans.	Non-gran. percentage	Polymorphos.	Eosinophils	Mast-cells
Nov. 17	·01	8,200	40	2	2·7	44·7	49·4	4·7	1
20	·02	11,650	43·8	1	1·5	46·3	47·1	4·8	1·6
23	·04	12,550	38·8	3·6	3·6	46	48·4	4·4	·9
27	·1	10,166	39·2	3·6	4·2	46	46·3	5·8	·8
30	·2	11,800	42	3·2	3·2	48·4	46·4	4	1
Dec. 4	·5	9,300	36·2	3·3	2	41·5	52·3	4·4	1·7
7	1	10,100	38·5	3·7	1·8	44	49	5·3	1·4
11	2	7,850	45·6	2·1	1·0	48·7	47·6	2·6	·8
15	4	10,050	32·1	5·2	4·5	41·8	53·1	3·8	1
19	8	18,000	15·6	1·6	4·9	22·1	75·5	4·2	1
20	—	12,700	29·6	4·1	3·0	36·7	57·8	1·2	·9
22	15	8,650	46·5	1·8	·8	49·1	47·1	3·1	·5
26	30	10,100	52·6	2·1	·3	55	43	1·1	·6
29	60	10,000	35·8	3·6	3·2	42·6	55	1·8	·7
Jan. 1	120	8,600	33·9	2·0	1·1	37	60	2	·9
4	250	10,000	34·9	3·4	2·4	40·7	55·4	2·2	1·5
8	500	8,300	35·9	1·9	·3	38·1	58·1	2·7	1
9	—	13,750	19·2	·4	0	19·6	79·2	1·1	0
10	—	19,250	17·6	1·9	2·1	21·6	71·8	1·9	1·6
11	—	14,250	26·6	4·2	·7	31·5	65·3	2·6	·4
12	500	8,150	39	2·2	·1	41·3	56·5	1·9	·1
16	800	11,650	36	4	·5	40·5	55·1	3·7	·5
17	—	11,100	15	1·3	·8	17·1	79·4	2·9	·4
18	—	15,800	21·8	2·8	1·7	26·3	70·1	3	·4
19	—	12,800	18·2	2·8	1·6	22·6	74·5	2·4	·3
23	1000	8,400	31·6	3·7	1·8	37·1	59·2	2·7	·9
24	—	19,200	9·9	1·8	·3	12	87·3	·1	·6
25	—	17,100	13·5	·7	3·4	17·6	80·2	1·9	·1
26	—	13,600	28·6	·9	·3	29·8	66·4	2·8	·8
27	—	8,400	42·8	2·5	2·1	47·4	48·1	3·9	·4
29	—	9,250	33·8	2	1·2	37	57·5	5·3	·1
30	—	12,750	30·7	1·2	2	33·9	61	4·3	·7
31	—	8,600	26·9	3	3·3	33·2	62·2	3·9	·5
Feb. 1	—	10,500	33	2·3	1·6	36·9	58·5	3·8	·5
2	—	14,000	31·5	3·8	2·7	38	55·1	5·6	1·1
5	1000	7,150	42·5	3·4	2·1	48	47·6	3·8	·5
6	—	15,500	18·3	·6	·6	19·5	78·6	1·1	·7
7	—	20,300	23·7	1·2	·9	25·8	69·3	3·6	1·1
8	—	12,250	32·5	1·2	1·2	34·9	59·5	3·7	1·8
9	—	8,700	42·4	·4	·9	43·7	47·6	5·7	2·7
10	—	8,500	43	1·8	1·3	46·1	48·9	3·8	1·1

Polynucleosis was also marked on Dec. 29, Jan. 1, 4, and 8. After the injections of 500 c.c., 800 c.c., and 1000 c.c. (*bis*), the blood was examined every day as was done in the case of massive injections in the goat. It will be seen that each of these injections was followed by an enormous rise in the polynuclear percentage although the hyperleucocytosis never exceeded 20,000.

Below are appended the absolute values of the non-granular and polynuclear cells on the days following these massive injections.

After the intravenous injection of 1000 c.c. toxin on March 12, the blood was also examined and it was found that on the day following, the polynuclear percentage had risen to the enormous value of 91.1 while the mononuclear percentage fell to 8.9. On the 14th of March, the polynuclear percentage fell to 83.9 while the mononuclear rose to 15.1.

Date	Non-granulars	Polynuclears	Toxin
Jan. 8	3162	4822	500 c.c.
9	2603	10823	
10	4032	14208	
11	4402	9230	
12	3321	4536	
16	4640	6380	800 c.c.
17	1887	8769	
18	4108	11060	
19	2816	9472	
23	3108	4956	1000 c.c.
24	2304	16704	
25	2907	13680	
26	3944	8976	
27	3948	4032	
29	3404	5244	
30	4191	7747	
31	2838	5332	
Feb. 1	3780	6190	
2	5320	7700	
5	3408	3337	
6	2945	12190	
7	5075	14007	
8	4270	7198	
9	3741	4089	
10	3910	4165	
Intravenous injection.			
March 12	4300	5200	1000 c.c.
13	1665	16835	
14	2940	16464	



When we consider the absolute values of the non-granular and polynuclear cells detailed above, it will be evident that the leucocyte response is mainly a polynuclear one. The total mononuclears fall suddenly, but rapidly regain their normal level, while the polynuclears rise slowly to a maximum and then slowly decline. From the fluctuations in the eosinophile and mast-cells it is impossible to draw any reliable inferences.

*Summary and Conclusions.*

It must be clear then from the above data on the horse and goat that the inoculation of diphtheria toxin produces a definite leucocytic reaction of polynuclear type. In fact the detailed evidence speaks more clearly in favour of a leucocytic reaction than Besredka's own results on the goat which he immunised. The question then arises: How are we to interpret the contradictory conclusions reached by Nicolas and Courmont? Metchnikoff (1905), while referring to the work of these authors in his *Immunity in Infectious Diseases*, maintains that a slight leucocytic reaction is apparent from their own figures and especially from the figures obtained in the early hours succeeding an injection. Of course, Nicolas and Courmont considered these slight changes to be negligible.

It seems to me, that the reason must be sought in the method of immunisation employed.

Nicolas and Courmont immunised their animals with very small doses many times repeated, and by such a method it was doubtless possible to reduce local swelling to a minimum. With no local reaction it would seem quite reasonable to expect an absence of general leucocytic reaction, but, as Metchnikoff maintains, such local tumefaction is never absent in horses which are subjected to increasing doses of toxin and which ultimately develop high grade antitoxin.

Throughout the immunisation of the animals detailed in the foregoing paper local swelling and oedema invariably followed the injections except in the earlier stages of immunisation when small doses were being given.

There is no doubt that, in order to produce a high degree of immunity in an animal, the stimulation must be sufficient. A certain small degree of immunity may always be reached without much leucocytic change, as was apparent in the early stages of "Plug's" immunisation.

It must be admitted, however, that, though the leucocytic reaction may be extremely marked after large doses of toxin, it does not necessarily follow that the antitoxin development will be correspondingly influenced.

Such a phenomenon was apparent in the case of "Plug," a relatively refractory horse.

In the more responsive horses, a parallelism between leucocytic reaction after large doses and increased antitoxin development would certainly be expected, without, however, inferring a causal relationship between these two phenomena. We cannot therefore admit the general applicability of Nicolas and Courmont's views, which merely indicate that cells subjected to small oft-repeated doses lose their power of reaction.

On the other hand there is probably no justification for the criticism brought forward by Besredka with regard to the results of Nicolas and Courmont, viz. that in the absence of antitoxin development, an absence of leucocytic reaction was to be expected.

We may therefore conclude that the leucocytic reaction of polynuclear type which follows the injection of large and increasing doses of diphtheria toxin is merely an evidence of efficient cell-stimulation and may not necessarily be accompanied by increased antitoxin development.

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# ON THE OCCURRENCE OF TOXIC COMPOUNDS OF TETANUS TOXIN AND ANTITOXIN, TETANUS TOXIN AND BRAIN EMULSIONS<sup>1</sup>.

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## *Introduction.*

EHRlich had not long announced his theory of immunity, when Wassermann and Takaki (1898) published their discovery, that the brain matter of the guinea-pig was capable of neutralising tetanus toxin. This was taken as a demonstration of the existence of Ehrlich's receptors in the cells of the normal brain.

Wassermann's strongest argument for the chemical nature of the combination between tetanus toxin and brain matter is its specificity.

A certain parallelism has been demonstrated to exist between the susceptibility and the toxin-neutralising property of the brain of an animal. Thus the cerebral cortex of highly susceptible animals, man and horse (Wassermann) and mouse (Metchnikoff) is strongly antitoxic, the brain of the less susceptible, rabbit, pigeon and fowl (Wassermann and Metchnikoff), has a feebler action, whereas in the case of the highly refractory tortoise and frog, the brain has no neutralising power.

Wassermann and Takaki thought at first, that brain and toxin

<sup>1</sup> This research was carried out in the laboratory of the Serum Department of the Lister Institute. To the Bacteriologist-in-charge of that department, Dr Dean, I am deeply indebted for inspiration and kindly criticism.

combined in the proportions which give the neutral mixture, just as on Ehrlich's theory a neutral mixture of toxin and antitoxin is one in which the combining affinities of both substances are satisfied. Metchnikoff (1898), however, pointed out that it was not necessary to conclude that the toxin was neutralised by the brain emulsion in the same way as by antitoxin. Metchnikoff, Roux and Salimbeni (1896) had shown that leucocytes could destroy cholera toxin, which they ingest while it is still retained within the bodies of the vibrios. Vibrios and toxin together are digested by the phagocytes. On the other hand a corresponding dose of toxin extracted beforehand from the vibrios, kills the test animal rapidly. Metchnikoff therefore argues, that the toxin in Wassermann's experiment may be absorbed physically by the solid particles of brain, and in this state may be taken up and digested by phagocytes.

Besredka (1903), went into the matter more thoroughly and showed that whatever the nature of the reaction, brain matter can combine with very much more toxin than it can neutralise. Besredka saturated the brain matter with a large excess of toxin, and then washed the combination in a centrifuge six times, till the washings only produced slight tetanus in a mouse, in a dose of 20 drops. One drop of the sediment of this brain matter was however sufficient to produce rapidly fatal tetanus in a mouse. The neutral mixture does not therefore represent a saturated chemical compound.

From the above brief outline of the literature of the subject it is evident that the nature of the combination between tetanus toxin and brain substance, is still open to discussion.

The main problems with which we have to deal are whether this combination is specific and whether it belongs to the same order of phenomena as the combination between antitoxin and toxin.

It seemed possible that a minute study of the quantitative combining relations under different conditions between brain substance and antitoxin respectively with tetanus toxin might throw further light upon the nature of their interaction.

The present paper records such a comparative study of the reaction between antitoxin and toxin, with that between brain and toxin.

In comparing a brain emulsion with an antitoxic serum, we are not really comparing a solid with a solution. The brain particles contain water, and, chemically considered, are equivalent to an emulsion of a colloid. Antitoxin is associated with the globulin of serum, and W. B. Hardy (1905) has shown that the transition from solution to precipitate, in the case of globulins, is not to be considered as a change

of state. The colloidal particles in the "solution" contain water and are bounded by a surface, but are too small to cause interference with a beam of light, and are easily held in suspension in water. The particles of the "precipitate" contain less water, and are larger, and therefore are visible, and sink to the bottom of the test tube. The difference between the clear "solution" and the flocculent "precipitate" is one of degree, not of kind.

The actual conditions under which tetanus toxin is absorbed by brain emulsion and antitoxic serum respectively are not therefore so dissimilar as might be supposed at first sight, and as shown by Danysz (1902) and Bordet (1903) some of the phenomena of toxin-antitoxin union suggest that this action is of the nature of absorption rather than that of combination between a strong (Ehrlich) or a weak (Arrhenius and Madsen) acid and base.

*The relation of toxin absorbed by brain substance to original toxin concentration.*

It seemed probable that the amount of toxin fixed by an emulsion of brain, would be some function of the amount of toxin originally present. This is the case, as may be shown by placing brain matter in the presence of varying quantities of toxin, centrifuging and estimating the amount of toxin remaining free in the supernatant fluid. By subtracting the observed free toxin from the known quantity originally present, we deduce the amount which has been fixed by the brain.

The following notation is used in the account of experiments:

—, =, ≡, denote increasing degrees of severity of local tetanus.

×, general tetanus, the animal certainly about to die within a few hours. Such animals were killed.

+, found dead.

The toxin used was prepared in April 1906. It was the filtrate from a 10 days' growth of tetanus bacilli in beef bouillon, previously freed from sugar by the growth in it of colon bacilli. It was preserved in the dark, at 10° C. under toluol.

This toxin was tested on mice on May 8th and October 22nd 1906, with the result given in Table I. It will be seen that the toxin lost a little in the five months interval.



TABLE I.

Dose per gram weight	Result after days given					
	1	2	3	4	5	6
May 8, 1906						
·000005	0	—	≡	≡	≡	≡
·00001	0	=	≡	+		
·00002	0	≡	≡	+		
·00004	0	≡	+			
·0001	0	×				
Oct. 22, 1906						
·000015	0	—	=	+		
·00003	0	=	≡	+		
·00006	—	≡	+			
·00012	=	+				

*Experiment I.* October 15th. Six tubes were charged with increasing doses of toxin; saline was added to make up the volume to 4 c.c. To each tube was added 2 c.c. of a 20 per cent. emulsion of guinea-pig's brain. The mixtures were allowed to stand for 11 hours at 10° C. and then a sample was removed from each tube and centrifuged. The supernatant fluid was tested for toxicity with the result given in Table II. In this table one mouse is selected for each tube, from a series all of which had different doses.

TABLE II. *Experiment I.*

October 16, 1906.

Total toxin	Toxic units lethal for 1 gm. of mouse in each .024 c.c.	Total brain	Dose of super- natant fluid per gm. weight of mouse	Date and result										Toxic units found in each .024 c.c. of supernatant fluid
				17th		18th		19th		20th	21st	22nd		
				M.	E.	M.	E.	M.	E.	M.	M.	M.		
.3 c.c.	20	.4 gm.	.048 c.c.	0	—	=	=	≡	≡	≡	≡	+	$\frac{1}{2}$	
.6	40	.4	.024	0	0	=	≡	≡	≡	≡	≡	+	$\frac{1}{4}$	
1.1	73	.4	.048	0	=	≡	≡	×					$\frac{1}{2}$	
1.6	107	.4	.024	0	—	≡	≡	+					1	
2.4	160	.4	.012	0	—	≡	≡	+					2	
4	267	.4	.003	0	—	≡	≡	+					8	

*The relation existing between the toxin found free in solution,  
and that combined with the brain matter.*

In my experiments I have chosen as the unit of volume, not the whole contents of the test tube, but ·024 c.c. because the test mice had a dose of this volume, or some multiple or whole fraction of it, for each gram body weight. The toxic unit, in which I have expressed the

quantities of toxin, is the amount per gram weight necessary to kill a mouse in 3 days (cf. Table I.). The unit of brain matter is the mass contained in  $\cdot 024$  c.c., that is  $\cdot 0016$  gram.

I have plotted the results in Fig. 1 where the ordinates represent the observed free toxin, and the abscissae the amount of toxin fixed by unit mass of brain matter. In the same figure I have plotted a curve calculated from Arrhenius and Madsen's equation for the reaction between toxin and antitoxin. It will be seen that the reaction between

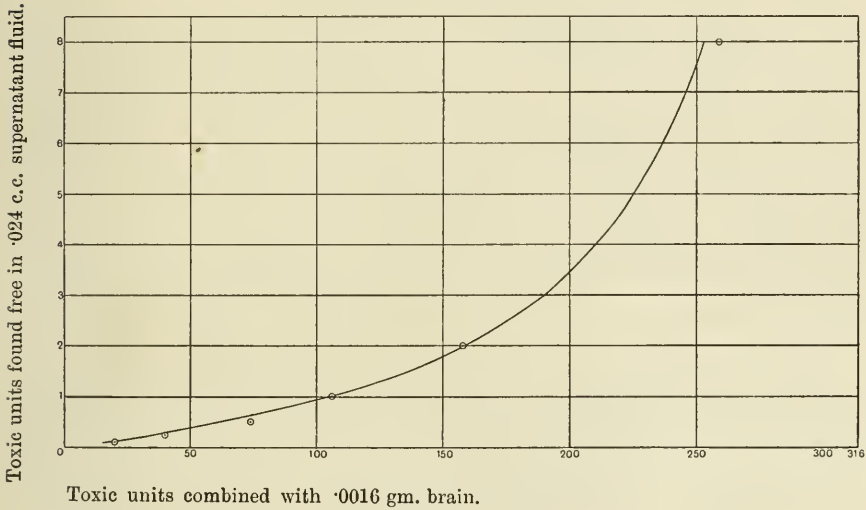


Fig. 1.

toxin and brain may very well follow the same law. The limit of error in the experiment is wide, so that I would not insist further on this agreement, but I hope to follow out this line of inquiry by a more accurate method.

The calculated and observed figures are given below :

Free toxin in $\cdot 024$ c.c.	Observed combined toxin	Calculated combined toxin
1/8	20	18·6
1/4	40	35
1/2	72	63
1	106	105
2	158	158
8	259	253

The form of equation used was

$$\left(\frac{\text{Free toxin}}{\text{volume}}\right)\left(\frac{\text{Uncombined brain}}{\text{volume}}\right) = K \frac{\text{Combined brain toxin}}{\text{volume}}.$$

The volume being unity may be neglected.

The dissociation constant,  $K$ , was found to be 2, and the amount of toxin equivalent to unit mass of brain (·0016 gram) was found to be about 316 toxic units.

Now whether we accept Madsen's or Bordet's view of the reaction between toxin and antitoxin, toxic combinations between the two must exist, comparable to Besredka's "cerveau toxique." These combinations will be formed whenever a toxic mixture of toxin and antitoxin is prepared. Their recognition, however, is difficult, because the presence of free toxin is a necessary condition of their formation. In spite of this they have been isolated in the case of ricin and antiricin. The combinations of ricin and antiricin under favourable circumstances form a precipitate, and Danysz (1902) found that, when ricin was present in excess, the precipitate contained practically all the ricin. He concludes justly that toxin and antitoxin "impregnate each other in variable proportions."

One cannot separate the compound of tetanus toxin with its antitoxin by the centrifuge, but one can remove the free toxin by allowing it to combine with brain matter, and centrifuging. The combined toxin and antitoxin then remain in the solution. In this way I have isolated toxic combinations of tetanus toxin with antitoxin, exactly comparable to Besredka's "cerveau toxique" and the toxic precipitate of Danysz.

In this series of experiments an antitoxin was used of which ·0032 c.c. mixed with 1 c.c. of my toxin gave a slightly toxic mixture. This was tested at the concentration at which the experiments were to be carried out. The amounts given in Table III. were diluted so that the volume in each tube was 8 c.c.; and mice received ·024 c.c. per gram body weight.

To show the combination of antitoxin with an excess of toxin above that contained in the neutral mixture, I chose a dose of antitoxin (·00016 c.c.) which would neutralise ·04 c.c. of toxin.

Test tubes were charged with ascending doses of toxin, beginning in the first tube with the  $L$  + dose for the above quantity of antitoxin.

The volume in each tube was made up to 4 c.c. with ·85 per cent.

salt solution. The antitoxin contained in 2 c.c. of the same salt solution was then added, and the mixtures were set at 10° C. for some hours (Ta in the tables). After this time, 2 c.c. of a brain emulsion was added to each tube, and the series set aside again for a time (Tb) which was allowed for the brain matter to combine with the free toxin present. Then a sample from each tube was centrifuged, and the clear fluid tested on a mouse.

TABLE III.

September 3, 1906.

Toxin	Antitoxin	Result and date						
		4	5	6	7	8	9	10
·1 c.c.	·00024 c.c.	0	≡	≡	+			
·1	·00028	0	≡	+				
·1	·00032	0	0	—	—	survived		

October 4, 1906.

Toxin	Antitoxin	Result and date						
		5	6	7	8	9	10	11
·7 c.c.	·0016 c.c.	—	+					
·6	·0016	0	≡	+				
·5	·0016	0	=	≡	+			
·4	·0016	0	0	0	0	—	=	

In each experiment a control series was set up, in which no antitoxin was used. The same doses of toxin were diluted to 6 c.c.; and 2 c.c. of the same brain emulsion were added to each tube, immediately after the brain had been added in the antitoxin series. Samples from the control series were centrifuged immediately after the similar samples from the antitoxin series.

The test dose per gram weight of mouse was ·024 c.c. in every case.

TABLE IV. *Experiment II.*

August 30, 1906.

Ta=21 hrs. at 10° C. Tb=35 mins. at 36° C.

Toxin	Antitoxin	Brain	Date and result							
			31	1	2	3	4	5	6	7
·05 c.c.	·00016 c.c.	·3 gm.	·	—	—	—	—	—	—	—
·1	·00016	·3	·	—	≡	≡	≡	≡	×	
·2	·00016	·3	·	=	≡	+				
Control Series.										
·05	0	·3	·	0	0	0	—	—	0	0
·1	0	·3	·	0	—	—	—	—	0	0
·2	0	·3	·	—	≡	≡	≡	≡	≡	≡

*Tetanus Toxin*TABLE V. *Experiment III.*

September 4, 1906.

Ta=15½ hrs. at 10° C. Tb=½ hr. at 36° C. and 2 hrs. at 10° C.

Toxin	Antitoxin	Brain	Date and result			
			5	6	7	8
·05 c.c.	·00016 c.c.	·2 gm.	0	0	0	0
·07	·00016	·2	0	0	0	—
·1	·00016	·2	0	0	—	≡
·14	·00016	·2	0	—	≡	≡
·2	·00016	·2	0	=	≡	+

## Control Series.

·05	0	·2	0	0	0	0
·07	0	·2	0	0	0	0
·1	0	·2	0	0	0	0
·14	0	·2	0	0	—	—
·2	0	·2	0	0	—	—

TABLE VI. *Experiment IV.*

September 17, 1906.

Ta=15 hrs. at 10° C. Tb=6 hrs. at 10° C.

Toxin	Antitoxin	Brain	Date and result						
			18	19	20	21	22	23	24
·05 c.c.	·00016 c.c.	·2 gm.	0	0	0	0	0	0	0
·07	·00016	·2	0	—	=	≡	≡	≡	×
·1	·00016	·2	0	0	—	=	=	=	=
·14	·00016	·2	0	=	≡	≡	≡	≡	+
·2	·00016	·2	0	=	≡	≡	≡	+	

## Control Series.

·05	0	·2	0	0	0	0	0	0	0
·07	0	·2	0	0	0	0	0	0	0
·1	0	·2	0	0	0	0	0	0	0
·14	0	·2	0	0	0	0	0	0	—
·2	0	·2	0	0	—	—	—	—	+

TABLE VII. *Experiment V.*

October 4, 1906.

Ta=20 hrs. at 10° C. Tb=5 hrs. at 10° C.

Toxin	Antitoxin	Brain	Date and result			
			5	6	7	8
1 c.c.	·0016 c.c.	·2 gm.	0	+		
2	·0016	·2	0	+		
4	·0016	·2	≡	+		

## Control Series.

·5	0	·2	0	=	≡	+
1·5	0	·2	—	≡	+	
3·5	0	·2	=	+		



TABLE VIII. *Experiment VI.*

June 19, 1906.

	Toxin made up to ·5 c.c.	Antitoxin made up to 2·5 c.c.	Brain made up to 2·5 c.c.	Date and result					
				20	21	22	23	24	25
Ta=0	·0625 c.c.	·0001 c.c.	·05 gm.	0	mouse lost				
	·25	·0001	·05	0	≡	≡	+		
Ta=32'	·0625	·0001	·05	0	0	0	0	0	0
	·25	·0001	·05	—	≡	≡	+		
Ta=90'	·0625	·0001	·05	0	0	0	0	0	0
	·25	·0001	·05	0	=	+			
Ta=270'	·0625	·0001	·05	0	0	—	—	=	≡
	·25	·0001	·05	0	+				

TABLE IX. *Experiment VII.*

June 11, 1906.

	Toxin made up to ·5 c.c.	Antitoxin made up to ·5 c.c.	Brain in emulsion in 1 c.c.	Date and result					
				12	13	14	15	16	17
Ta=0	·0125 c.c.	·00002 c.c.	·01 gm.	0	0	0	0		
	·025	·00002	·01	0	0	0	0		
	·05	·00002	·01	0	≡	≡	+		
	·1	·00002	·01	=	+				
Ta=6 hrs.	·0125	·00002	·01	0	0	≡	≡	≡	+
	·025	·00002	·01	0	=	+			
	·05	·00002	·01	0	=	≡	+		
	·1	·00002	·01	—	≡	+			

In all cases the antitoxin had combined with so much toxin, that the fluid in each tube of the antitoxin series was much more toxic than the fluid in the corresponding control tube.

The results are given in Tables IV., V., VI., VII.

In the last experiment (Exp. V., Table VII.) the amount of toxin in the control series was not the same as that in the antitoxin series, but this amount less by the  $L +$  dose.

The long time taken by toxin and antitoxin to enter into combination is well illustrated by this method. In Exp. VI. four series of tubes were prepared, in which the time allowed for the combination of toxin and antitoxin varied from zero to  $4\frac{1}{2}$  hours. Table VIII. shows the result. It is only after a considerable time that our toxic compound has become stable enough to resist dissociation by the brain emulsion. Table IX. refers to a similar experiment.

*Dissociation of brain-toxin compound.*

Besredka (1903), in the paper already quoted, shows that the brain-toxin combination can be completely dissociated by means of antitoxin, and infers that antitoxin has a greater affinity for toxin than has the brain matter. Even so, both brain and antitoxin may owe their power of combining with toxin to the same side-chain. Organic chemistry is full of instances, where the affinities of a side-chain are altered in degree and not in kind by alterations in the radicle to which they are attached; instance the various chlor-acetic acids.

Besredka's experiment was as follows: To his "cerveau toxique" he added antitoxin and allowed the mixture to stand. The brain matter was then washed in the centrifuge, till the washings were free from antitoxin. It was then tested and found not only to be atoxic, but to have regained completely its protective power. The toxin had all gone over into combination with antitoxin. He used, however, an excess of antitoxin, though he does not state exactly how much.

*Experiment VIII.*

I repeated the experiment in a modified form, using a minimal quantity of antitoxin. The amount of antitoxin chosen was '00032 c.c. the *L* + dose for this quantity being '1 c.c. of my toxin. '1 c.c. of toxin was therefore mixed with '2 gram of brain, in emulsion in 2 c.c. of saline, and the mixture was made up to 4 c.c. In a second tube '2 c.c. of toxin and '2 gram of brain were used. The mixtures were left for two hours at 10° C. The toxin remaining in solution in tube (1) would then be nearly nil, and in tube (2) considerably less than the amount required to kill a mouse in a dose of the volume generally used (*vide* Table V., control series).

To each tube was then added '00032 c.c. of antitoxin, contained in 4 c.c. of saline, and the tubes were left for 8 hours at 10° C.

Now if the antitoxin has lost any of its protective power, it must have taken the toxin from combination of the latter with the brain matter; also there is enough toxin present to neutralise the whole of the antitoxin, so that, if the solution retains any protective power, we know that the brain matter has been able to hold in combination some of the toxin, in spite of the presence of unsaturated antitoxin.

The contents of the two tubes were centrifuged, and the supernatant fluids tested for antitoxin. It was found that unsaturated antitoxin was

present in each. Volumes of 1 c.c. were mixed with different quantities of toxin. Now we started with '00032 c.c. antitoxin in each tube, and the volume in each tube was 8 c.c. Each cubic centimetre therefore corresponds to '00004 c.c. of antitoxin, for which the  $L_0$  and  $L +$  doses of my toxin are respectively '01 and '0125 c.c. The tests are given in Table X. It will be seen that the fluid of tube (1) protected against '005 c.c. of toxin, therefore that half the antitoxin was unsaturated with toxin, and that the brain matter had been able to retain in combination more than half the toxin which was given to it originally.

We cannot, therefore, argue that the combination of toxin with brain is of a different nature from the combination with antitoxin, on the ground that an excess of antitoxin can take all the toxin from the brain, to which it was previously bound. The toxin is divided between the brain and the antitoxin, and it is only in the presence of excess of the latter that increased mass-action enables it to take over all the toxin.

TABLE X. *Experiment VIII.*

October 4, 1906.

Fluid of tube (1)	Toxin (in 1 c.c.)	Date and result						
		5	6	7	8	9	10	11
1 c.c.	'005 c.c.	0	0	0	0	0	0	0
1	'0075	0	0	0	—	≡	≡	×
1	'01	0	0	≡	≡	≡	×	
1	'0125	0	≡	≡	≡	+		
Fluid of tube (2)								
1	'005	0	0	—	≡	≡	≡	≡
1	'0075	0	—	≡	≡	≡	≡	+
1	'01	0	≡	≡	+			
1	'0125	—	≡	+				

Moreover, there is already a large body of evidence showing that dissociation of a toxin-antitoxin combination can occur.

Behring and Ransom (1898) demonstrated this in the case of tetanus toxin and antitoxin, by dilution. Using a strong toxin, they prepared a mixture with antitoxin which was nearly neutral. The test mouse had very slight tetanus. This mixture was diluted 10 times, 100, 1000, and 10,000 times and the same volume of each dilution was injected into the test mice. The thousandth dilution caused death in three days, and all the dilutions were more toxic than the original mixture. If this mixture is allowed to stand for 24 or 48 hours before the dilutions are prepared, the effect is not obtained. The toxicity decreases gradually

as the dilution increases. After this time, therefore, the combination has become more stable.

R. Otto and Hans Sachs (1906) have lately confirmed these results.

Carl Bruck (1904), working with Wassermann, showed that if a toxin-antitoxin mixture be injected subcutaneously in the site of a previous injection of adrenalin, its toxicity is greater than in a control animal. He explains the result by stating that the antitoxin is forced to remain at the site of the inoculation, because of the vascular constriction, while the toxin which diffuses more readily, and travels along nerves, is removed from the sphere of influence of the antitoxin. He regards the experiments as a proof that dissociation occurs, and to a greater extent in the adrenalin animal than in the control; but it may be objected that the free toxin, in the absence of a normal circulation, is held at one spot in greater concentration, and is hence able to invade the axis-cylinders in larger quantities than when it is diffused through the body by the blood and lymph streams.

Finally J. A. Craw (1905 and 1906), following up Martin and Cherry's (1898) work with gelatine filters, succeeded in demonstrating the dissociation of a toxin-antitoxin compound *in vitro*. He used Myatherium lysin and antilysin, and passed neutral mixtures of the two through gelatine filters, under a pressure of 100 atmospheres. The antilysin has the larger molecules, or particles, and is held back by the filter while some of the toxin is forced through. A toxic filtrate is obtained.

The correspondences between brain and antitoxin in their reactions with tetanus toxin are seen to be fairly complete, but there remains the fact, that when we add brain emulsion to toxin, we do not obtain nearly so sharp a neutralisation point as we do on adding antitoxin. Exp. IX. (Table XI.) is an example of this. 1 c.c. of a 2 per cent. dilution of toxin was placed in each of 4 tubes. Different weights of brain, always emulsified in 1 c.c. of saline were added to each tube. Four

TABLE XI. *Experiment IX.*

October 22, 1906.

Toxin	Brain	Weight of guinea-pig	Date and result						
			23	24	25	26	27	28	29
·02 c.c.	·3 gm.	325	0	0	—	=	≡	≡	≡
·02	·16	330	0	—	=	≡	≡	≡	≡
·02	·08	325	0	=	≡	≡	≡	≡	≡
·02	·04	350	0	=	≡	≡	≡	+	

guinea-pigs were used as test animals, and each had a dose of .2 c.c. from a different tube. Though .04 gram of brain was enough to delay death till the sixth day, .3 gram was not enough to prevent altogether the onset of tetanus.

I am inclined to believe that this phenomenon is to be explained by the destruction of the brain particles within the tissues. Toxin might thus be set free, in the same way as ricin can be set free from its combination with fibrin by the aid of digestive ferments (Martin Jacoby, 1902).

### *Conclusions.*

(1) The affinity of brain matter for tetanus toxin is specific, as is that of antitoxin.

(2) A solution of pure toxin is easily rendered innocuous by treatment with brain matter; but if a small dose of antitoxin has been added to the toxin some hours beforehand, treatment with brain matter no longer suffices to render the solution atoxic. The free toxin is removed, and we have isolated a toxic compound of toxin and antitoxin.

(3) Both brain-toxin combinations and antitoxin-toxin combinations dissociate with more or less rapidity, unless in the presence of enough free toxin (and free brain or antitoxin) to maintain the state of equilibrium. Consequently to obtain a neutral mixture one adds a large excess of brain or antitoxin beyond the combining equivalent. The dissociation is thus reduced till free toxin is no longer recognisable by our tests.

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## THE TREATMENT OF MEDITERRANEAN FEVER BY MEANS OF VACCINES, WITH ILLUSTRATIVE CASES.

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FOR years we have been vainly striving to find some method of treatment capable of preventing or cutting short the relapses so constantly present in Mediterranean fever. Though certain drugs have for a time seemed successful, their beneficial effects are usually uncertain and transient, so that the treatment of the disease has come to be almost entirely symptomatic. Many germicides have been tried; among these "cyllin" has lately been said to shorten the course of the fever. In my hands this has not been the case. Burney Yeo's chlorine and quinine mixture has here occasionally produced very satisfactory results when given with a rising temperature, apparently cutting short a wave of pyrexia, which from experience we had expected to last ten days or more; but its action is very uncertain.

In this disease a constant intoxication is going on, due to the presence of a specific micro-organism which may apparently flourish in the internal organs for years, rapidly increasing at any favourable opportunity when presumably vitality is lowered, and giving rise to fresh attacks of pyrexia. During these recurrent periods of fever the specific organism is easily isolated from the peripheral blood.

Failing an effective antitoxin, the most successful and rational line of treatment would seem to be the employment of means for increasing and stimulating the "phagocytes" rendering them able to combat this influx of toxin-producing germs. Unfortunately, as I have shown (1902), one of the most constant features of the disease is a relative reduction of the number, as well as the phagocytic activity, of the polymorphonuclear white cells.

In the hope of increasing the number of these polymorphonuclear leucocytes, fresh yeast was given in milk, daily for a month, to the patients in a ward occupied by cases of Mediterranean fever, but with no marked success, and without beneficial effect on the temperature. Koch and Petruschky employed vaccines prepared from the bacilli of tuberculosis and typhoid fever in the treatment of these diseases, and Wright has recently extended and elaborated this method of treatment, having shown that vaccines prepared from the causal organism of a disease, when injected in suitable quantities, are capable of stimulating the body to produce protective substances which are thrown into the blood and modify the clinical manifestations of the disease.

Staff-surgeon S. T. Reid, R.N., in 1905, by applying Wright's method to a small number of cases of Mediterranean fever (9) was apparently so successful that an extensive trial of this treatment was considered desirable in the wards at Haslar, where the cases of this disease are fairly numerous. This trial has now been made during the last eight months on almost all the cases, to the exclusion of any active drug treatment.

In every case the dosage and the nature of the reaction was calculated by recording the opsonic index, as devised by Wright and Douglas (1904). At the same time the amount of the agglutination reaction was also determined.

#### *Preparation of the Vaccine.*

A freshly isolated strain of the *Micrococcus melitensis* was used. The culture was taken during life from the spleen of a patient in this hospital.

Agar cultures of ten days' growth were employed, the organisms being scraped off and emulsified in 100 c.c. of distilled water. The emulsion was heated to 60° C. for half-an-hour, and after 0.5% carbolic acid had been added, sealed up and kept as a stock solution. After twenty-four hours 10 c.mm. of the vaccine were spread on an agar tube to make sure of its sterility.

#### *Estimation of the strength of the Vaccine.*

1. *By opacity.* A sample of the vaccine was run out into a square glass bottle, giving a thickness of fluid of 1.5 cm. This just obscured 0.5 Snellen's type.

2. *By dilutions and culture.* By means of a measuring pipette, 5 c.mm. of the freshly prepared emulsion were diluted 50 times, and this again on three occasions, giving a dilution of 1 in 6,250,000. Five c.mm. of this high dilution were plated out, and showed on the fifth day 150 colonies of the *Micrococcus melitensis*, which works out at 937,500,000 organisms per c.mm., or 187,500,000 for each c.mm. of the vaccine.

3. *By weight of the dried organisms.* 20 c.c. of the freshly prepared emulsion, after heating, but before the addition of the carbolic acid, were run off into a long tube. The estimation was kindly undertaken by Dr C. J. Martin, F.R.S., who found that the 20 c.c. of vaccine contained 4 mg. of dried *Micrococcus melitensis*, or 0.2 mg. per c.c.

#### *Method of injection.*

About 10 c.c. of the stock vaccine were periodically run off into a sterile bottle with two rubber caps. 0.5 c.c. doses were drawn up into freshly made glass tubes by means of a sterile teat, and then the tubes were sealed in the flame. These were taken to the ward for use, where each dose was expressed into a sterile glass capsule as required.

A 1 c.c. all-glass sterilised syringe was employed for injecting the vaccine. The injections were made in the region of the loin, the part having been prepared with spirit soap, ether and biniodide solution.

#### *Method of noting the effects of the vaccine by the opsonic indices.*

Blood from all the cases under treatment in the ward was periodically taken at the same time, at first twice a week and later once a week, and the opsonic index estimated. A few cases were examined daily, the results of these cases being given in the special charts.

My own leucocytes were used throughout for the estimation. The plasma was removed and the cells were well washed four times in 0.9% normal saline solution. A freshly prepared weak emulsion of a young agar culture of *M. melitensis* was made for each operation. One part of the patients' unheated serum, one part of my washed blood cells, and a half part of the fresh emulsion, were then well mixed and drawn up into small tubes, sealed, and placed in the incubator at 37° C. for 20 minutes. This mixture was then blown out on to slides, films were made, and these, when dry, were fixed in alcohol for ten

minutes, and stained with carbol-toluidin blue for half-an-hour. The opsonic indices of the serum of six surgeons under instruction were ascertained, and as the average of their sera was found to be identical with the index of my own serum, the latter for reasons of convenience, was assumed to be normal, and was employed as the control in the tests.

At least, twenty consecutive normal polymorphonuclear leucocytes were generally counted in each film and the average number of ingested cocci taken.

#### *Agglutination reactions.*

Besides testing the serum for the opsonic index, capillary tubes were put up on each occasion with the dilutions 1 in 20, 1 in 200, and 1 in 2000, to demonstrate the quantity of agglutinins present. In the cases that were examined daily, the frequency with which the dilutions were made was much greater, as is seen in the special charts.

#### *Frequency of the injection of the Vaccines.*

The vaccines were generally given at ten day intervals, unless the clinical symptoms contraindicated their use.

#### *Number of cases and injections given.*

Altogether 224 injections in 61 cases have been given here since the commencement of the treatment. Beyond a certain amount of temporary tenderness in the loin, there were no unpleasant local effects, except some heat and redness in two cases which passed off in twenty-four hours. Occasionally there was a slight rise in the temperature indicating some general reaction. In one case the temperature shot up to 103.6° F. with a slight shiver and headache. In the majority of cases nothing was noted. Most of the men believed strongly in the efficacy of the injections and asked for their repetition; in no case was the vaccine given if the patient raised any objection.

#### *Records of cases treated.*

In this paper I have tried to record as far as possible the general clinical characters of the cases under treatment with the marked features in each, and in the charts are shown the temperature, opsonic, and agglutination curves. It is essential to follow the course of fever both



before and after the administration of the vaccines if any deductions are to be drawn, and as the cases lasted over such long periods, I have with this object in view reduced the charts into small dimensions.

### Epitome of Cases.

#### 1. *Severe Undulant type.*

*No. 1. Chart 7.* W. E. G. Severe case with high continued type of fever, followed by slight undulations. Vaccines were administered commencing in the 6th week, and were repeated for six weeks without appearing to do any good, they were therefore stopped, but two further doses were given in the 24th and 25th weeks. The opsonic index, which had remained about normal, rose considerably after the two last doses of the vaccine. The agglutinins were very high throughout (1 in 2000).

*No. 3. Chart 8.* V. H. A severe continued type of fever passing into the undulant form. The first vaccines were given in the 12th week. The opsonic index at first rose, then fell rapidly; vaccines stopped. After the next relapse, three injections were given, there being a normal morning temperature; this was followed by a further wave of pyrexia, in spite of a high opsonic index. He however gained two stones in weight and felt much stronger during this four week relapse. He was discharged on sick leave. His agglutinins rose and remained high (1 in 2000).

*No. 5. Chart 9.* C. N. A long undulant type of fever. Vaccines were commenced in the 33rd week of the fever and were given weekly. During the treatment he had three relapses, the last being the most severe. The vaccines were powerless to prevent the reinfections, though the opsonic curve steadily rose. The agglutinins fell. He was invalided in the 42nd week.

*No. 7. Chart 10.* P. A. Admitted for phthisis. Fever of an undulant type with great diurnal variations, (there was cough etc., no tubercle bacilli in sputum). Vaccines were commenced in the third week, repeated about every ten days with apparently no effect on the temperature. After a final severe wave he made steady improvement and gained weight rapidly. The opsonic index though high slowly fell during the long fever, rising again when the temperature was normal. The agglutinins were at first high, fell low during the course of the disease, but rose again in convalescence.

No. 10. *Chart 11.* J. W. Severe undulant type of fever. Vaccines were commenced in the 11th week. The opsonic index rose after the second injection, the temperature fell after the fourth, and remained down, with improvement in nutrition and power. The agglutinins remained very low. The case was finally invalided.

No. 11. *Chart 12.* T. L. Severe undulant type of fever. Vaccines were commenced in the 10th week, a wave of pyrexia having started. This aborted, and there was no further rise. His general condition rapidly improved, gaining 25 lbs. in 21 days. The opsonins rose and the agglutinins were fairly high.

No. 12. *Chart 13.* J. M. Late severe undulant type of fever. Vaccines were commenced in the 33rd week, when a wave had well started, the temperature suddenly fell, remaining down. The opsonic index mounted very high, but the agglutinins were low. This was a brilliant result for the time.

### 2. *Mild Undulant types.*

No. 21. *Chart 14.* B. C. Undulant type of fever. Vaccines were commenced in the 6th week, when he had a marked purpuric eruption over the body. Four injections were given, his opsonins rose very high and the temperature fell. The agglutinins were moderately high. He steadily gained weight and was discharged cured.

No. 22. *Chart 15.* T. B. Late undulant type with neuritis. Vaccines were commenced in the 22nd week. There was no further rise in the temperature and the opsonic index steadily rose.

### 3. *Intermittent types of fever.*

No. 46. *Chart 16.* H. H. Intermittent type of fever. Vaccines were commenced in the 16th week. Eight injections were given, which had apparently no effect on the fever. The opsonic index made an irregular but gradual ascent, the agglutination curve steadily fell. The patient was invalided.

No. 47. *Chart 17.* G. G. Slight irregular intermittent fever, with neuritis and effusion into the left knee. Vaccines were commenced in the 26th week. The opsonins rose high and the temperature fell. He gained weight and his general condition improved markedly.

It is not possible to give the details of all the cases referred to, or the charts compiled from them, I have therefore made a short summary of the main facts relating to them.

*Severe undulant types of fever.* In numbers 2, 4, 6, 8, and 15 relapses were not prevented, and the vaccine did not appear to influence the course of the fever for good. In most of them the opsonic index was very variable, though there was a tendency generally for it to rise in convalescence. Numbers 9, 16, and 17 all showed marked improvement in the course of the temperature, opsonic index, and weight.

*Mild undulant types.* In numbers 19, 20, 28, 30, 31, 34, and 35 the temperature fell almost at once, and both opsonins and agglutinins rose; in most there was a rapid gain in weight, as much as 14 lbs. in 14 days in one case.

In numbers 18, 23, 26, 29, 33, 37 the temperature fell more slowly, and the opsonic index rose; but the agglutinins remained low. All were discharged to duty.

Case No. 25 was a relapse after one year's apparent freedom from the fever. Vaccines appeared to lower his temperature and hasten his recovery.

Case No. 27 was peculiar in that each injection caused the temperature to rise, on one occasion to  $103.6^{\circ}$ , but the man steadily improved.

Case No. 32 was of rather special interest. It was an irregular undulant type of fever in an oldish man. During a slight wave of pyrexia one injection of 0.3 c.c. of the vaccine was given, but it caused such marked local and constitutional reaction for a few hours that he refused any further. For 14 days the opsonic index steadily fell, then slowly rose and remained above normal. It is difficult to say how long the one dose of vaccine influenced this curve. The general symptoms slowly improved, but the agglutinins were very low throughout.

In case No. 36 vaccine was given in the 11th week, the temperature abruptly dropped on the second day, but there was no rise in the opsonic index.

*Intermittent type of fever.* In case No. 48, the temperature fell, the opsonic index rose, and general improvement was marked.

In case No. 49 although the opsonic index rose, there was no marked improvement in his general condition, and his agglutinins kept constantly low.

The total of 47 cases show an improvement in the temperature curve in 28 cases.

## VACCINE RECORD.

## Table of cases treated.

0.25 c.c. equals 468,750,000 *M. M.* or 0.05 milligram of dried *M. M.*

No. of case	Name	Amounts injected	No. of injections	Subsequent course	
				Temperature	Opsonins
1.	W. E. G.	.2 c.c., .4 c.c., .25 c.c., .25 c.c., .2 c.c., .2 c.c., .25 c.c.	7	no improvement	rose at last.
2.	A. R. M.	.25 c.c., .25 c.c., .3 c.c., .25 c.c., .25 c.c.	5	do.	rose.
3.	V. H.	.25 c.c., .4 c.c., .25 c.c., .4 c.c., .25 c.c., .25 c.c.	6	do.	rose at last.
4.	W. M.	.25 c.c., .3 c.c., .25 c.c.	3	do.	fell.
5.	C. N.	.3 c.c., .3 c.c., .2 c.c., .2 c.c., .3 c.c., .2 c.c., .5 c.c. <sup>1</sup> , .25 c.c., .3 c.c.	9	do.	rose.
6.	A. H.	.3 c.c., .3 c.c., .5 c.c., .5 c.c., .25 c.c., .3 c.c., .25 c.c., .4 c.c., .3 c.c.	9	do.	irregular.
7.	P. A.	.2 c.c., .5 c.c., .3 c.c., .3 c.c., .25 c.c., .25 c.c., .5 c.c., .2 c.c., .25 c.c.	9	do.	steadily fell then finally rose.
8.	G. B.	.25 c.c., .25 c.c., .3 c.c., .25 c.c., .3 c.c.	5	fell	rose.
9.	C. L.	.25 c.c., .25 c.c., .25 c.c., .25 c.c., .25 c.c.	5	fell	rose.
10.	J. W.	.1 c.c., .3 c.c., .2 c.c., .25 c.c., .3 c.c., .3 c.c.	6	fell	rose.
11.	T. L.	.2 c.c., .2 c.c., .25 c.c., .3 c.c., .3 c.c.	5	fell	rose.
12.	J. M.	.2 c.c., .3 c.c.	2	fell	rose.
13.	J. G. B.	.3 c.c., .3 c.c., .5 c.c.	3	fell	rose.
14. } 39. }	T. N.	.3 c.c., .3 c.c., .3 c.c., .2 c.c., .25 c.c., .3 c.c., .25 c.c., .25 c.c., .3 c.c.	9	no improvement	irregular.
15.	G. W. M.	.2 c.c., .25 c.c., .3 c.c., .25 c.c., .5 c.c., .25 c.c., .25 c.c.	7	do.	fell.
16.	G. V.	.2 c.c., .3 c.c., .25 c.c., .5 c.c.	4	fell	steady.
17.	W. C.	.25 c.c., .2 c.c., .25 c.c., .3 c.c., .2 c.c., .2 c.c., .25 c.c., .25 c.c.	9	no improvement	rose slightly.
18.	A. T.	.3 c.c., .3 c.c.	2	fell	rose.
19.	H. D.	.2 c.c.	1	fell	rose.
20.	F. B.	.2 c.c.	1	fell	rose.
21.	B. C.	.25 c.c., .25 c.c., .25 c.c., .25 c.c.	4	fell	rose.
22.	T. B.	.25 c.c., .25 c.c.	2	fell	rose.
23.	E. J.	.25 c.c., .25 c.c., .25 c.c., .3 c.c., .3 c.c.	5	no improvement	rose slightly.
24. } 43. }	H. F. C.	.25 c.c., .25 c.c., .25 c.c., .3 c.c.	4	fell	rose.
25.	F. M. W.	.25 c.c., .25 c.c., .1 c.c., .4 c.c.	4	improved	rose slightly.
26.	F. H.	.25 c.c., .25 c.c., .25 c.c., .3 c.c., .3 c.c., .5 c.c.	6	do.	very regular.
27.	T. D.	.25 c.c., .25 c.c., .25 c.c., .25 c.c. <sup>2</sup> , .3 c.c.	5	no improvement	rose.
28.	W. H.	.25 c.c., .25 c.c., .25 c.c., .3 c.c., .25 c.c.	5	improved	rose.
29.	J. P.	.25 c.c.	1	kept low	rose.
30.	J. I.	.25 c.c., .25 c.c.	2	kept low	rose.
31.	T. F.	.25 c.c., .25 c.c., .25 c.c.	3	fell	rose.
32.	H. P.	.3 c.c. <sup>3</sup>	1	no improvement	fell for 14 days then rose.
33.	H. H.	.3 c.c., .3 c.c., .3 c.c., .2 c.c., .4 c.c., .25 c.c., .25 c.c.	7	do.	regular.
34.	A. W.	.3 c.c., .2 c.c., .25 c.c.	3	do.	rose.
35.	J. S.	.25 c.c., .5 c.c.	2	fell	steady.
36.	D. G.	.2 c.c., .4 c.c.	2	fell	rose slightly.
37.	J. W.	.25 c.c., .3 c.c., .25 c.c.	3	fell	rose.
38.	W. S.	.25 c.c., .25 c.c., .25 c.c.	3	fell then relapsed	rose for a short time.
40.	Mr S.	.25 c.c., .25 c.c.	2	no improvement	rose.
41.	F. T.	.25 c.c.	1	fell	rose.
42.	Mr C.	.25 c.c.	1	fell	rose.
44.	H. B.	.25 c.c., .3 c.c., .35 c.c.	3	fell slowly	rose.
45.	H.	.25 c.c., .3 c.c.	2	fell	rose.
46.	H. H.	.3 c.c., .1 c.c., .2 c.c., .25 c.c., .3 c.c., .3 c.c., .3 c.c., .25 c.c.	8	no improvement	irregular rise.
47.	G. G.	.25 c.c., .3 c.c., .3 c.c.	3	fell	rose.
48.	G. F.	.25 c.c., .25 c.c., .25 c.c.	3	kept low	irregular.
49.	J. C.	.3 c.c., .3 c.c., .3 c.c., .3 c.c., .3 c.c.	5	no improvement	irregular.

<sup>1</sup> Marked local reaction for 24 hours.<sup>2</sup> Marked general reaction for 24 hours.<sup>3</sup> Evening temperature ran up to 103.6° F.

*General considerations in relation to the temperature.*

In Mediterranean fever the course of the temperature is very variable.

1. In some cases we may have a long wave of continued fever ranging from  $105^{\circ}$  to  $102^{\circ}$  F. lasting perhaps for 7 or 8 weeks, the condition of the patient closely simulating that seen in typhoid fever. After short apyrexial intervals, fresh waves of fever follow, each becoming as a rule shorter and less severe.

2. Cases where from the commencement the undulations of pyrexia are more moderate in intensity and duration, but pyrexia recurs over and over again, and the late relapses may be of any severity.

3. Cases which have lasted for months (4 to 12 or more) having an irregular intermittent type of fever, generally accompanied with neuralgic pains, chronic joint affections, and cachexia. In the majority of cases there is a great tendency for the waves of pyrexia gradually to die out as the duration of the disease extends.

*Importance of the temperature curve as an index of the clinical condition.*

No case of Mediterranean fever can be correctly understood without having a continuous temperature chart of the case to study. By following the course of the fever month by month a fair index is given of the intensity of the toxæmia from which the patient has suffered, and we gather a slight idea of the probable sequelæ.

*Effects of the Vaccine on the temperature record.*

In a large proportion of the cases, the immediate effect of the vaccine was not appreciable; in a few, as seen in charts 5 and 9, a slight rise occurred the same evening, and in case 32 the evening temperature rose to  $103.6^{\circ}$  F.

In case 12, chart 13, an injection was given on the fourth day of a relapse (33rd week), the wave was checked, the temperature fell rapidly and did not rise again.

In case 11, chart 12, the first injection was given on the third day of a rising wave in the 10th week of the fever; the wave stopped abruptly and there was no relapse.

In case 10, chart 11, after the fourth injection, a prolonged wave suddenly ended, and no relapse occurred.



Similar favourable influences on the temperature are seen in case 13, case 18, case 19, case 20, case 21 chart 14, case 22 chart 15, and cases 28, 31, 35, 36, and 37.

Out of these eleven last mentioned cases, ten were treated before or during the 13th week with the vaccine.

In cases 29, 30, 48, 49 the temperature at the time of giving the vaccine was low, and no subsequent rise took place.

It would appear that in mild undulatory relapses or with a chronic intermittent type of fever, the fall to normal is frequently accelerated, judging from previous experience in the treatment in so many cases.

In very acute cases with high fever no benefit could be observed either in the clinical symptoms or on the temperature curve. *Vide* case 1 chart 7, case 2, case 7 chart 10, and cases 14, 15, and 16.

The *prevention of relapses* is perhaps the greatest desire of any one treating this trying disease.

In the administration of vaccines we seemed to have a possible means of doing so. At the commencement of this investigation, some very successful cases led me to think that at last there was a reliable method. *Vide* charts 12, 13, 15. Further experience has proved this hope to be without foundation, for over and over again cases under treatment, when appearing to be doing favourably have suddenly developed another wave of pyrexia, not differing from those met with in cases otherwise treated. *Vide* charts 8 and 9.

#### *Relation of the temperature curve to the opsonic curve.*

In thirty of the forty-seven cases here charted which were treated with the vaccine, the opsonic index rose markedly in convalescence. In others who had not received any vaccine (see chart 6) or who had not had any for a prolonged period, the index ran high during severe pyrexial periods.

### **Agglutination Reaction.**

#### *Magnitude of agglutination reaction in cases of Mediterranean fever.*

In the paper on this subject by Birt and Lamb, *Lancet*, Sept. 9th, 1899, complete reactions were found in 6000 fold dilutions of the serum. In the present investigation, the maximum obtained and highest tried for was 1 in 10,000, chart 7. In some chronic cases with much cachexia it falls as low as 1 in 10.

*Course of the curve without Vaccines.*

In the above mentioned paper by Birt and Lamb, it was demonstrated that as a rule with acute fever the agglutination reaction was at first high, falling rapidly before death, or remaining fairly constant till convalescence set in, when it fell. In cases running a chronic course though at first high, it became low and variable. A rapid fall from a high level was a bad sign, if accompanied by acute symptoms, and a consistently low agglutination curve made prognosis unfavourable.

In my observations on the blood reaction in Mediterranean fever previously referred to, the same general characters were demonstrated, but it was noticed that after the fall in convalescence, the agglutinins again rose considerably in the cases which remained free from relapses.

Further experience has shown more clearly than before that in prolonged cases the agglutination reaction may only be obtained in very low dilutions. This occurred recently in a patient at Haslar, when for some months before death 1 in 10 was the highest dilution giving a complete reaction, though on the day before he died the *Micrococcus melitensis* was isolated from his blood. (*Med. Fever Com. Report Royal Society*, part 4, p. 104.)

*Duration of agglutination reaction.*

Birt and Lamb found a complete reaction in a 1 in 50 dilution after five years. I have not found any to exceed this period, but have frequently obtained good reactions in a 1 in 40 dilution after three years.

*Effect of the Vaccine on the agglutination curve.*

Charts 4 and 5 show a rise in the agglutination curve after the injections of the vaccines, generally attaining its maximum about the third day. In chart 4 the rise was very great, from 1 in 1000 to 1 in 10,000. In chart 5 after the first injection it rose to 1 in 6000, and after the second to 1 in 8000, but after the third there was very little response.

*Relation of the agglutination to the opsonic curve.*

The relationship of the two curves seems to vary very considerably. At one time it was believed that the registration of the agglutination curve would give a correct indication of the amount of protective substances in the blood, a view put forward by Wright (1902), and since held by others.

It has since been shown by French (1906), that the amount of agglutinins are not indicative of the amount of opsonins present.

On the addition of the emulsion of the *Micrococcus melitensis* to the mixture of normal washed cells and the serum of patients suffering from Mediterranean fever, we have to reckon with the possibility of the specific agglutinins causing clumping of the micrococcus and rendering their ingestion in large numbers into the phagocytes easier than normal. In those sera which show high agglutination values, as is often the case in the early stages of the disease with high fever, one would expect that clumping would be very marked. That this is so is evident in some of the stained films showing high opsonic indices, for not only are the phagocytes full of organisms, but the latter are seen distinctly agglutinated throughout the film. From the study of a very large number of films it is however evident that this expected agglutination is *rarely present* sufficiently to explain the high phagocytic index of the blood under examination.

In chronic cases, after the injection of the vaccine, there was a slight rise in the agglutinins quickly falling to the usual low level.

To test the relationship of the two curves the blood was examined daily for both agglutinins and opsonins, the results being shown in charts 1 to 6.

A critical examination of these, as well as of all the accompanying charts of cases extending over many weeks, shows that there is no real relationship between the agglutination and the opsonic curve. Many of those showing evidence of low agglutinins having a high opsonic index. These observations are more in conformity with the experiments of Leishman (1905), than with those of some other observers.

## Experiments relating to Opsonins in Mediterranean Fever, etc.

*Comparison of the amount of sensitising substances in heated and unheated serum.*

The serum was heated to a temperature of 60° C. for half-an-hour, then added to my washed blood cells, and finally to the emulsion.

		Average cocci ingested	Index
No. 1. <i>Acute Mediterranean fever case.</i>			
Unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	11	1·2
Heated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	9	1·0
My unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	9	1·0

The heated serum of the patient still contained substances favourable to phagocytosis.

No. 2. <i>Chronic Mediterranean fever case.</i>			
Unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	7	1·2
Heated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	2·5	·41
My unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	6	1·0

No. 3. <i>Chronic Mediterranean fever case.</i>			
Unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	10·2	·94
Heated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	2·8	·16
My unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	10·8	1·0

No. 4. <i>Chronic Mediterranean fever case.</i>			
Unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	7·2	1·2
Heated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	1·9	·33
My unheated serum	1 part		
My washed blood cells	1 part		
Emulsion	1 part	5·7	1·0

			Average cocci ingested	Index
<i>Controls.</i>				
No. 5. <i>Healthy surgeon.</i>				
	Unheated serum	1 part		
	My washed blood cells	1 part		
	Emulsion	1 part	13·6	1·04
	Heated serum	1 part		
	My washed blood cells	1 part		
	Emulsion	1 part	3·0	0·23
	My unheated serum	1 part		
	My washed blood cells	1 part		
	Emulsion	1 part	13·0	1·0
	My heated serum	1 part		
	My washed blood cells	1 part		
	Emulsion	1 part	2·6	·2

In all these last the sensitising substance was almost destroyed by heat.

*Experiment to ascertain whether the removal of the serum and replacing the content with normal saline solution would cause an equal diminution in the phagocytic action of the washed blood cells.*

			Average cocci ingested	Index
No. 1.	My washed blood cells	1 part		
	Normal saline solution	1 part		
	Emulsion	1 part	5	·37
	My washed blood cells	1 part		
	My unheated serum	1 part		
	Emulsion	1 part	13·5	1·0
No. 2.	My washed blood cells	1 part		
	Normal saline solution	1 part		
	Emulsion	1 part	2·9	0·78
	My washed blood cells	1 part		
	My unheated serum	1 part		
	Emulsion	1 part	3·7	1·0
No. 3.	My washed blood cells	1 part		
	Normal saline solution	1 part		
	Emulsion	1 part	2·2	0·5
	My unheated serum	1 part		
	My washed blood cells	1 part		
	Emulsion	1 part	4·0	1·0
No. 4.	My washed blood cells	1 part		
	Normal saline solution	1 part		
	Emulsion	1 part	7·0	·7
	My washed blood cells	1 part		
	My unheated serum	1 part		
	Emulsion	1 part	10·0	1·0

These results indicate that moderate phagocytosis still occurs in the absence of serum.



*Relative importance of the serum and of the leucocytes  
in phagocytosis.*

The following experiment was carried out to show the relative phagocytic values of the serum and the blood cells of a patient suffering from chronic Mediterranean fever, and that of normal blood mixed in different ways.

			Average no. in each PMN cell	
			Case 1	Case 2
1.	Patient's unheated serum	1 part		
	Patient's washed blood cells	1 part		
	Emulsion of living <i>M. M.</i>	$\frac{1}{2}$ part	23	46
2.	Patient's unheated serum	1 part		
	Normal washed blood cells	1 part		
	Emulsion	$\frac{1}{2}$ part	8.6	25
3.	Normal unheated serum	1 part		
	Patient's washed blood cells	1 part		
	Emulsion	1 part	16	30
4.	Normal unheated serum	1 part		
	Normal washed blood cells	1 part		
	Emulsion	$\frac{1}{2}$ part	19	29

The same mixtures with dead cultures gave proportionally similar results.

From the above it would appear that the specific serum with its own washed blood cells was more powerfully phagocytic than when mixed with blood cells from another individual, and that the specific serum with blood cells from an individual not suffering from Mediterranean fever was less active than when the patient's blood cells were mixed with non-specific serum. This seems to indicate that the increased phagocytosis was due in part to an increased capacity of the patient's white blood cells to ingest the organisms of Mediterranean fever.

These experiments as a whole demonstrate that though sensitising thermolabile substances for the *Micrococcus melitensis* are found in the serum in varying quantities, yet when these have been removed by heat, or by replacing the serum with normal saline solution, phagocytosis still takes place to a considerable extent.

It is possible, as suggested by Metchnikoff in his Harben Lectures, that "the absorption of the microbes may be effected without the help of opsonins, or that, should such help be indispensable, the opsonins may be supplied by the leucocytes themselves."

*The course of the opsonic curve in patients not treated by vaccine, or not having received any for long periods.*

It is very difficult in such a disease as Mediterranean fever to estimate how much of the variation observed in the opsonic index is due to the addition of small artificially introduced doses of the toxic microbe, when at any time fresh natural periods of intoxication may occur. To illustrate these variations in the index, charts of the opsonic curves of two patients in very similar conditions of fever, cachexia, and periods of illness were carefully recorded day by day. One (chart 5) being treated with vaccines from the first, the second (chart 6) only having the vaccine after a considerable time. In the latter, we found a slightly raised and irregular index, followed by a rather high curve preceding a fresh wave of pyrexia. When the vaccine was given there was a further rise in the opsonic index and a fall in the temperature.

Chart 8 shows also a high opsonic curve with a severe wave of fever.

*Effect of the Vaccine on the opsonic curve.*

Charts 1 to 6 show from daily observations the opsonic curves in Mediterranean fever cases (the remainder were estimated weekly).

Chart No. 1. After the first dose of the vaccine, there was a slight negative phase followed by a rapid rise, this positive phase lasting over a week. When it reached the normal line, a second dose was given, which produced no negative phase and only a slight positive one.

Chart No. 2. The first injection of vaccine was given at the end of the third month of the fever, in a very anaemic and cachectic case.

After the first injection there was a short well marked negative phase, and a slight irregular positive one. With a falling opsonic curve and a rising temperature a second dose failed to elicit any response (the case was severe and ran a long course).

Chart No. 3. These observations were taken after the fourth injection on the 55th day of the disease, the temperature being slightly irregular. The opsonins from a low level rose steadily reaching a maximum on the third day, falling to the normal line on the sixth.

Chart No. 4. The first dose of the vaccine was given in the 10th week, with an irregular temperature, the opsonic curve rose rapidly but was very irregular. A second rather larger dose was followed by a more marked response, and a fall in the temperature.

Chart No. 5. Vaccines were commenced about the end of the third month of the fever, which was of an undulant type. Three injections were given, the response after the first and third being very marked.

Chart No. 6. This case was admitted also about the end of the third month of the disease, and was used for a control case to show the variations in the opsonic index without the influence of vaccines, quoted before.

In the long charts the general effect of the vaccines is seen to be a constant raising of the opsonic index.

*Presence of a negative phase.*

Col. Leishman in his paper on anti-typhoid inoculations, *Journ. Hygiene*, Vol. v. 1905, is sceptical as regards typhoid fever of the well marked negative phase described by Wright. A study of these charts shows that this phase is frequently absent, or very short in cases of Mediterranean fever, especially when the initial reading was below normal, the rise being steady from the time of giving the vaccine.

*Relief of pain.*

In the more chronic cases, having the very common secondary nerve and arthritic pains so frequently met with, the vaccines appeared to produce at least temporary improvement in some instances.

*Gain in weight.*

The gain in weight which occurred in 50 per cent. of his cases is regarded by Staff-surgeon Reid as the most certain indication of the favourable action of the vaccine. In some of my cases it was remarkable, half to one pound a day; at the same time too much value must not be placed on this rapid gain in the body weight, as it has frequently been observed in cases otherwise treated, when convalescence first sets in.

Looking over the total series of cases, the points standing out most clearly are that:

1. The injection of the vaccine, if carried out with care, is productive of little or no local or general disturbance to the patient.
2. The injection of the vaccine in most instances produced a more or less steady rise in the phagocytic power of the blood, as registered by the opsonic index.
3. The earlier the vaccine is commenced the more favourable the result.

4. Though the vaccine is ineffective when given in high fever or with severe clinical symptoms, and is unable with certainty to prevent relapses, yet in many cases the fever undulations were curtailed, or reduced in severity after its administration.

In a disease like Mediterranean fever with a fatality of only 4 per cent. it is very difficult to provide adequate controls to check these results. The severity of an epidemic varies much from year to year, and even during each part of the year, so that comparisons are unsatisfactory.

I have however compared 60 cases treated with the vaccines in the early half of this year with a similar number from the same period last year, with the following result:

	Average duration in Haslar Hospital
60 cases 1905, without vaccines	67 days
60 cases 1906, with vaccines	75 days

Among the latter were some very severe cases, ten being over 100 days in the hospital, and one 245 days; some of these were months in the ward before the vaccines were commenced.

In the first series fourteen were over 100 days in the hospital.

### *Conclusions.*

The vaccine treatment of Mediterranean fever appears in a certain number of cases to produce a beneficial effect, the severity of the symptoms being diminished, the general condition improved, and the duration of the disease curtailed.

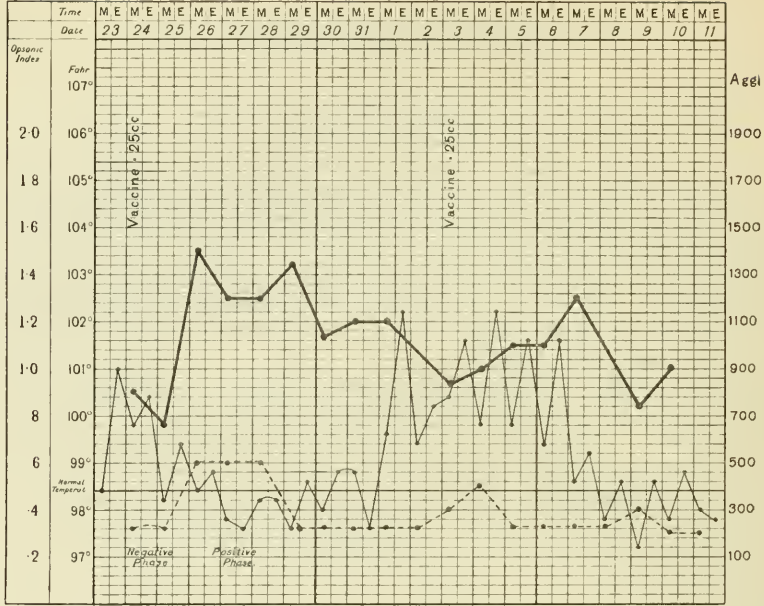
The disease being characterised by its irregular course, long latent periods and unexpected recrudescences, it is one in which the estimation of the value of any method of treatment is most difficult. Too great weight therefore cannot be put on the above impression.

In the more acute type of case with high fever and evidence of severe intoxication, the method appears to have a deleterious, instead of a favourable action.

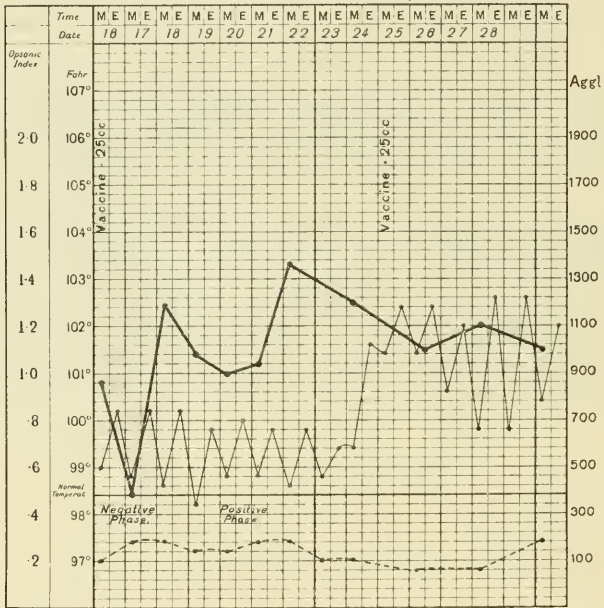
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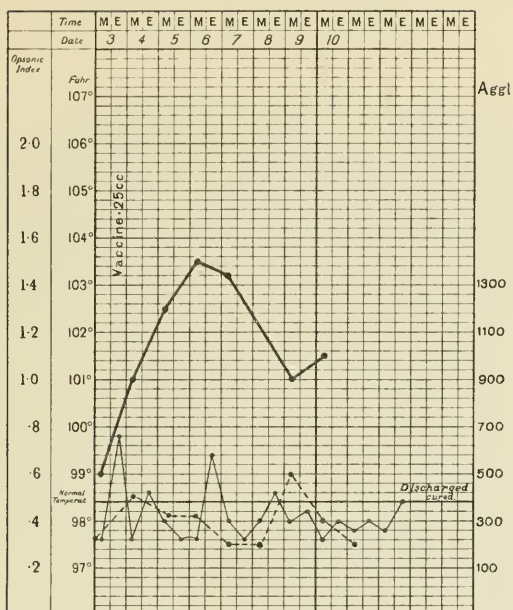




Thin line=Temperature curve. Thick line=Opsonic curve.  
Dotted line=Agglutinin curve.  
Chart No. 1. Case No. 38. 1st injection of vaccine in 11th week of the fever.



Thin line=Temperature curve. Thick line=Opsonic curve.  
Dotted line=Agglutinin curve.  
Chart No. 2. Case No. 40. Vaccine commenced end of 3rd month of fever.



Thin line=Temperature curve. Thick line=Opsonic curve.  
Dotted line=Agglutinin curve.

Chart No. 3. Case No. 41. 4th injection of vaccine 8th week of the disease.



Thin line=Temperature curve. Thick line=Opsonic curve.  
Dotted line=Agglutinin curve.

Chart No. 4. Case No. 43. Vaccine commenced in 10th week of fever.

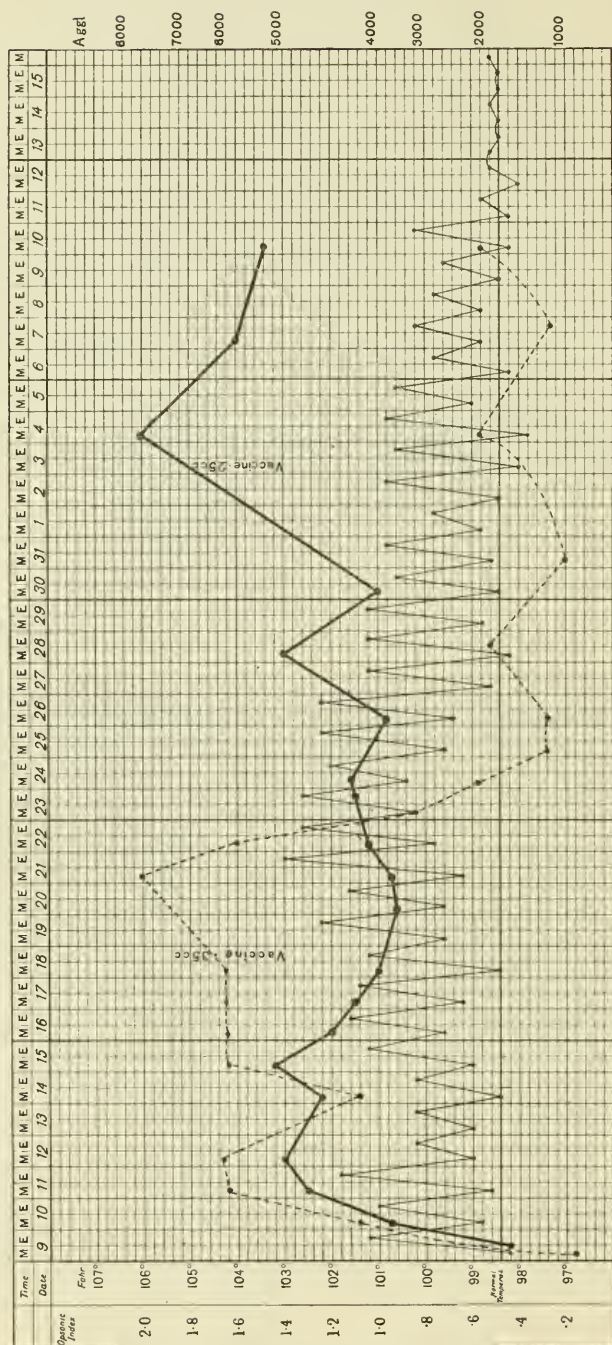
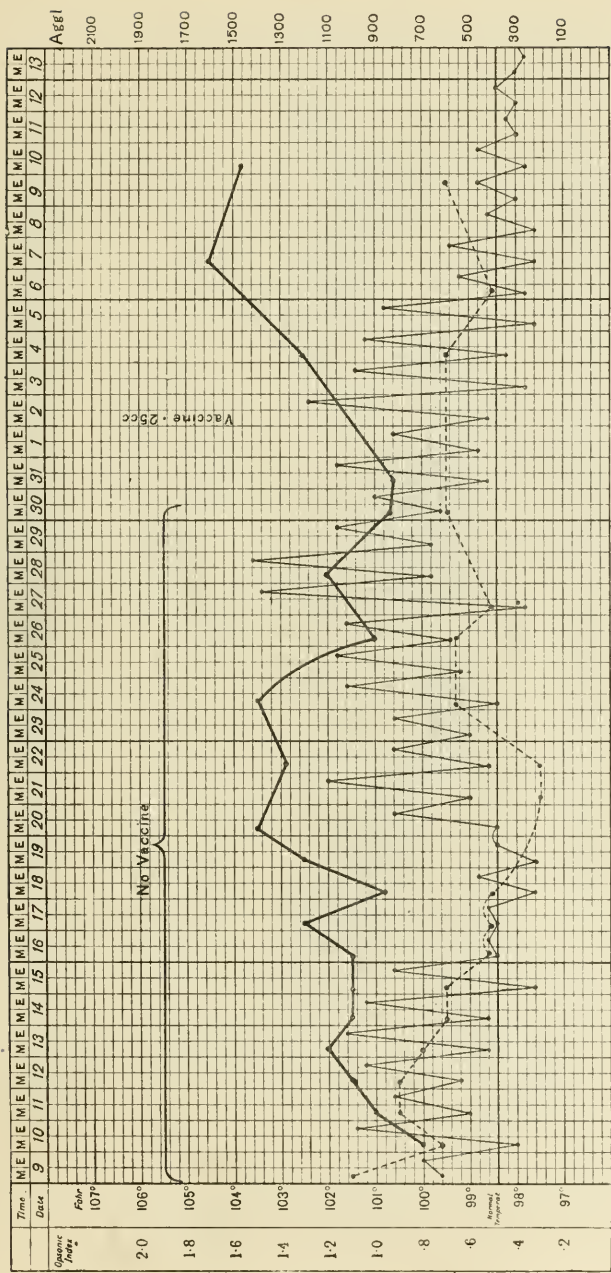
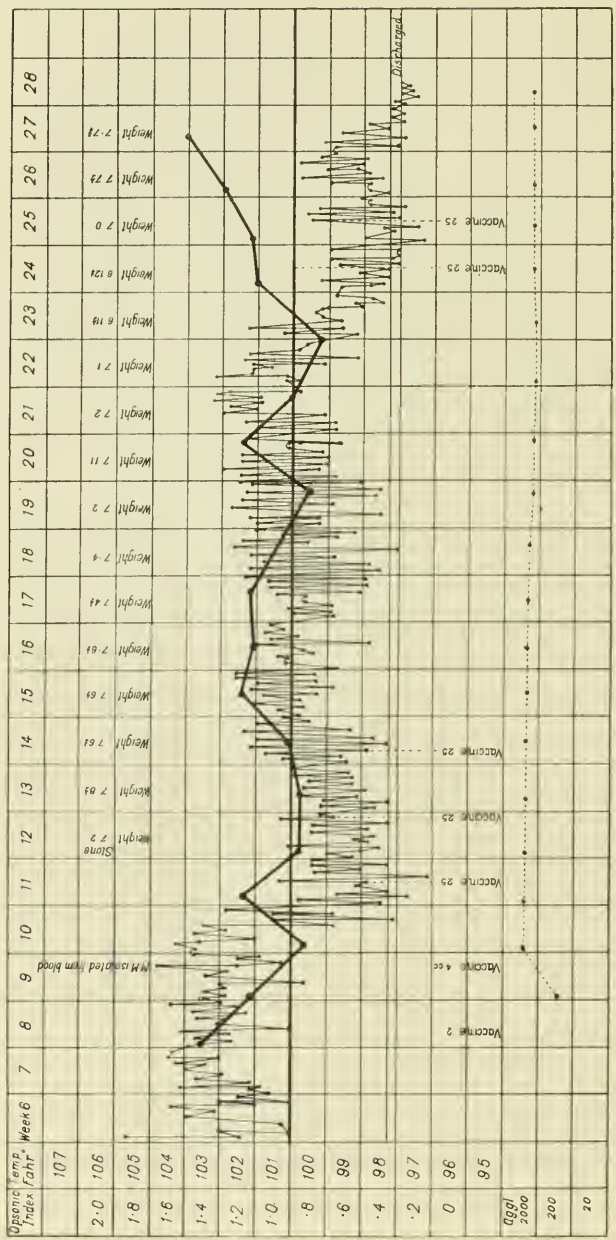


Chart No. 5. Case No. 44. Vaccine commenced middle of 3rd month of fever. N.B. Agglutinin curve is on a different scale to other charts.



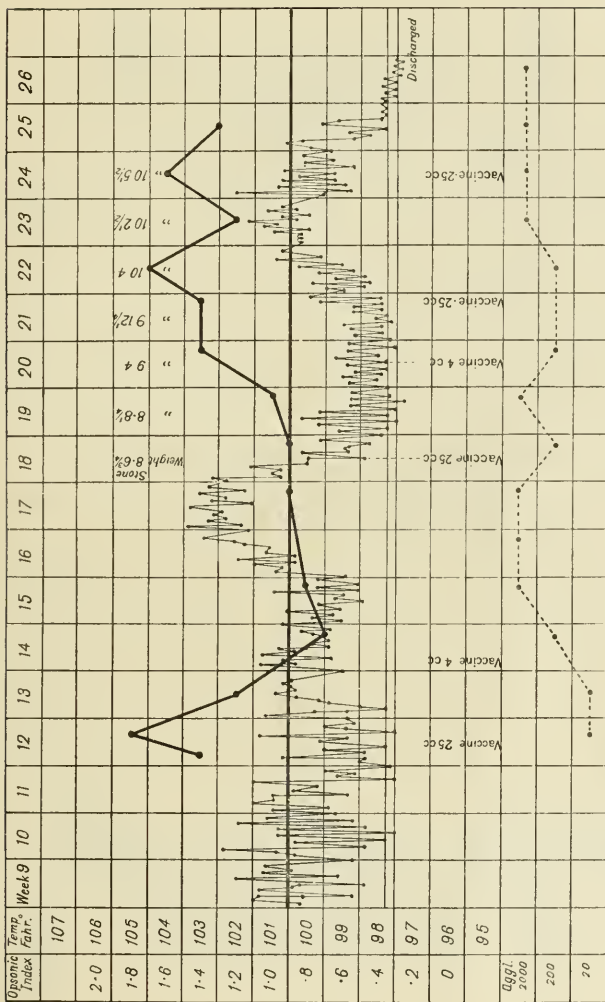


Thin line = Temperature curve.      Thick line = Opsonic curve.      Dotted line = Agglutinin curve.  
Chart No. 6. Case No. 45. Vaccine commenced 4th month of fever.

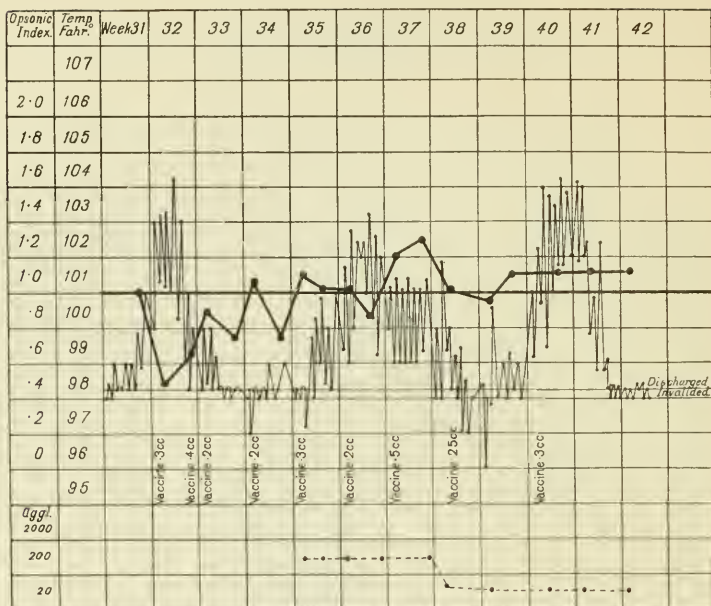


Thin line=Temperature curve. Thick line=Opsonic curve. Dotted line=Agglutinin curve.  
Chart No. 7. Case No. 1. W. E. G., 24, Stoker. Onset 3 March 1906. Discharged 14 September 1906.  
Invalidated for deafness.



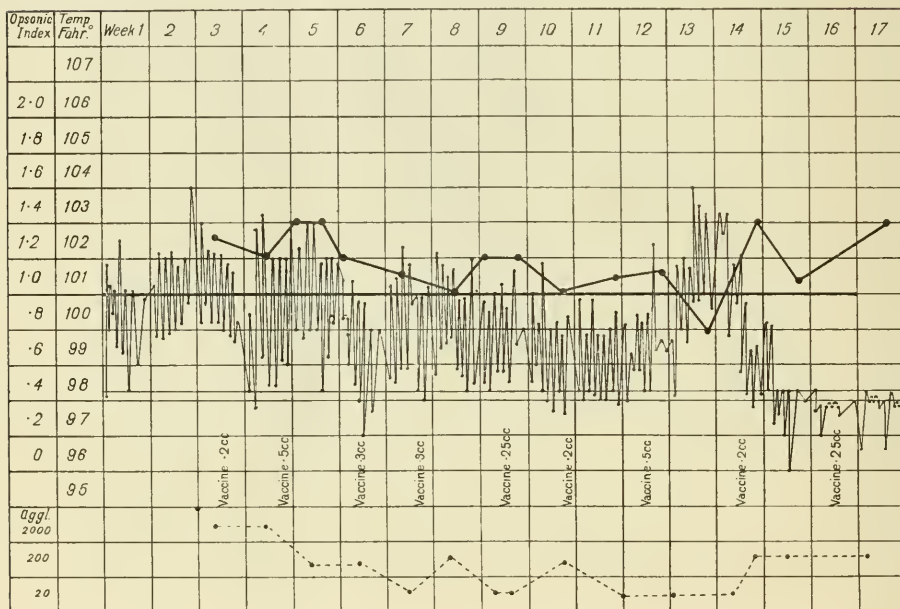


Thin line = Temperature curve. Thick line = Opsonic curve. Dotted line = Agglutinin curve.  
 Chart No. 8. Case No. 3. V. H., 38, Ch. Cook. Onset 3 February 1906. Discharged 11 August 1906.



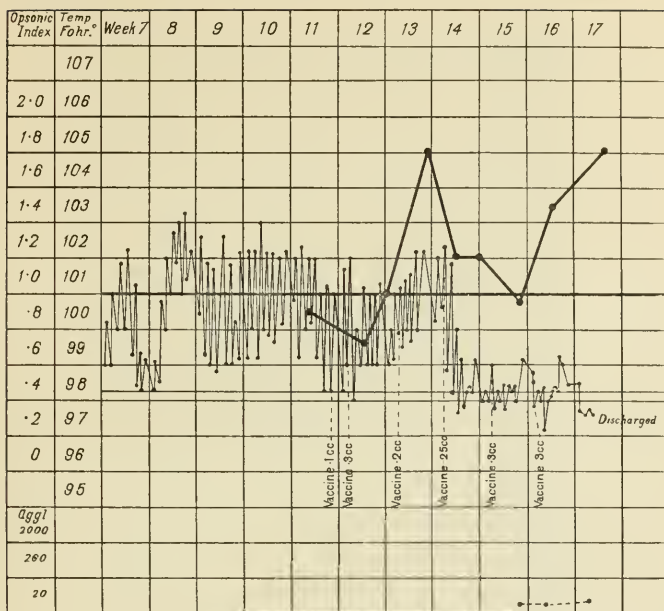
Thin line=Temperature curve. Thick line=Opsonic curve.  
Dotted line=Agglutinin curve.

Chart No. 9. Case No. 5. C. N., 17, Boy. Leviathan. Onset 5 July 1905.  
Discharged 17 April 1906. Invalided.



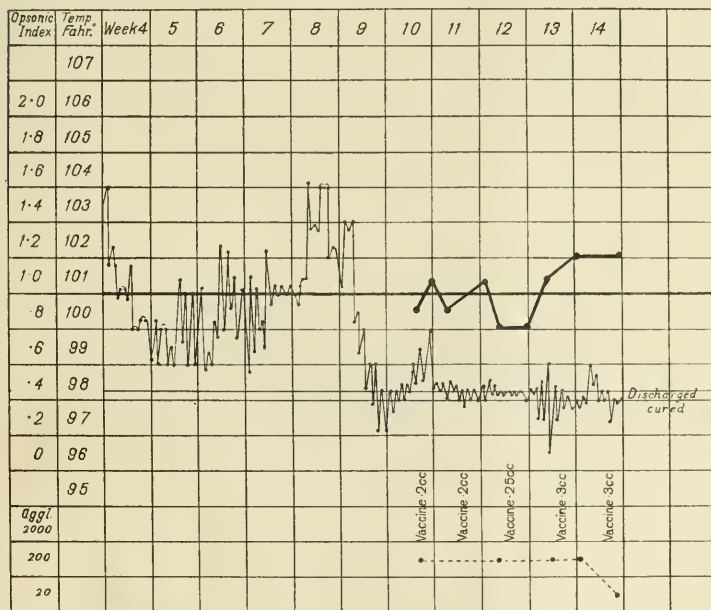
Thin line=Temperature curve. Thick line=Opsonic curve. Dotted line=Agglutinin curve.

Chart No. 10. Case No. 7. P. A., 23, Pte R.M.L.I., Egmont. Onset 28 November 1905.  
Discharged cured 12 June 1906.



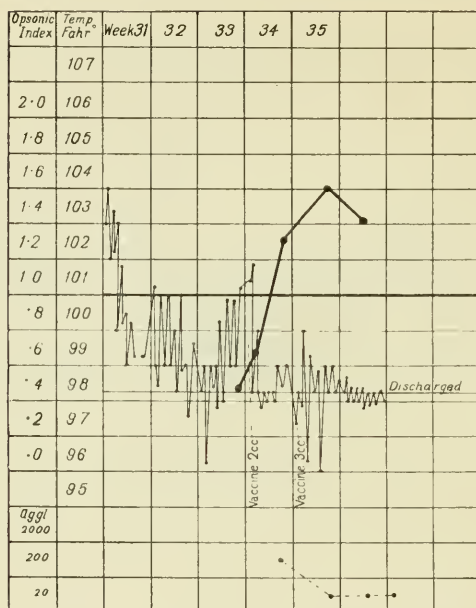
Thin line = Temperature curve. Thick line = Opsonic curve.  
Dotted line = Agglutinin curve.

Chart No. 11. Case No. 10. J. W., 28, 2nd S.B.S., Malta Hospital.  
Onset 14 November 1905. Discharged 11 March 1906.



Thin line = Temperature curve. Thick line = Opsonic curve.  
Dotted line = Agglutinin curve.

Chart No. 12. Case No. 11. T. L., 23, G.M.A. Onset 30 November 1905.  
Discharged 14 March 1906.

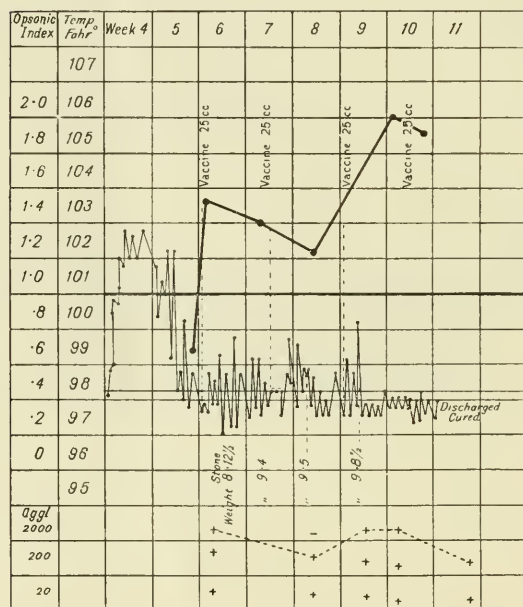


Thin line=Temperature curve.

Thick line=Opsonic curve.

Dotted line=Agglutinin curve.

Chart No. 13. Case No. 12. J. M., 25, Sto. Vernon. Onset 29 May 1905. 31. 1. 06.  
Discharged 12 March 1906.

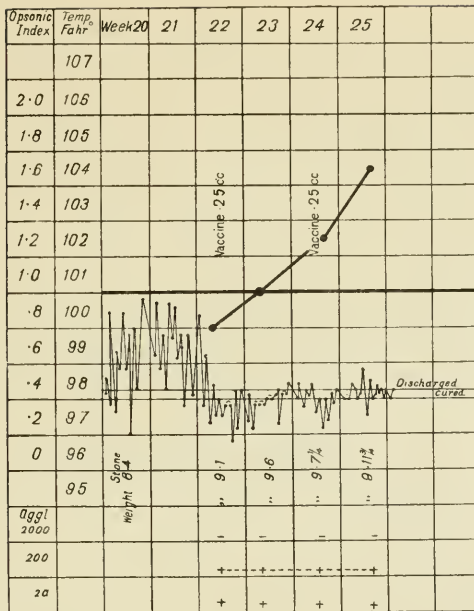


Thin line=Temperature curve.

Thick line=Opsonic curve.

Dotted line=Agglutinin curve.

Chart No. 14. Case No. 21. B. C., 28, Stoker. Onset 13 April 1906.  
Discharged 23 June 1906.

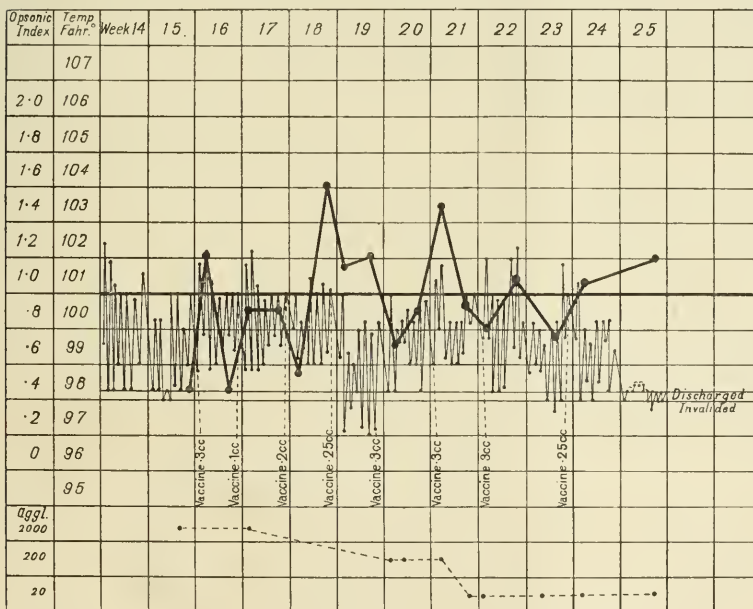


Thin line = Temperature curve.

Thick line = Opsonic curve.

Dotted line = Agglutinin curve.

Chart No. 15. Case No. 22. T.B., 25, Bandsman. Onset 19 December 1905.  
Discharged 7 June 1906. Invalided.



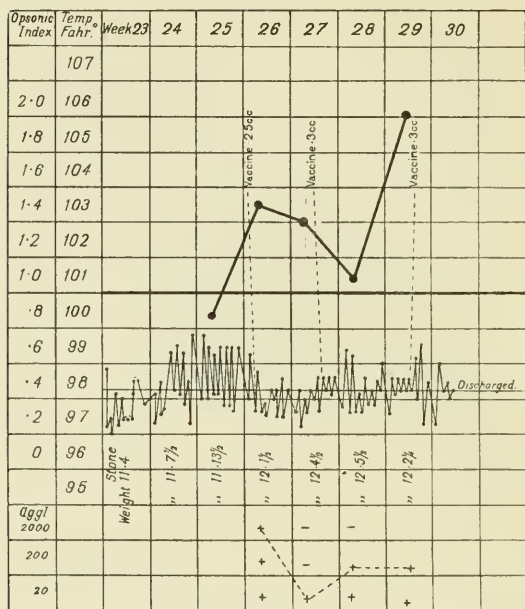
Thin line = Temperature curve.

Thick line = Opsonic curve.

Dotted line = Agglutinin curve.

Chart No. 16. Case No. 46. H.H. A.B., Vulcan. Onset 7 October 1905.  
Discharged invalided 9 April 1906.





# ON THE OCCURRENCE OF THE MICROCOCCUS CATARRHALIS IN NORMAL AND CATARRHAL NOSES AND ITS DIFFERENTIATION FROM OTHER GRAM-NEGATIVE COCCI.

By J. A. ARKWRIGHT, M.D.

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THE *Micrococcus catarrhalis* was first isolated by Kirchner (1890) from cases of influenza-like illness. Since then several epidemics of acute catarrh and bronchitis associated with this organism have been recorded.

The first epidemic recorded in this country was that described by Dunn and Gordon (1905) as occurring in Hertfordshire; the clinical aspect of the disease was that of an acute febrile illness with variable symptoms, of which sore throat, scarlatiniform eruptions, and symptoms suggesting meningitis were the most striking features. Other epidemics have been recorded by Ghon and Pfeiffer (1902), Bezançon and de Jong (1905), and others.

In a large proportion of the cases examined Gordon obtained the *Micrococcus catarrhalis* in great numbers and often in pure culture from the nose and throat. Several writers have described the occurrence of other Gram-negative cocci in the nose.

The Meningococcus (or *Diplococcus intracellularis meningitidis* of Weichselbaum) has been found in the naso-pharynx and nasal fossae, almost exclusively in cases of meningitis or in those in close contact with meningitis patients. A few occurrences have been, however, recorded apart from obvious meningitis infection by Schiff (1898), Weichselbaum and Ghon (1905), and others.

Dunham (1906) examined cultures from the nose and throat of 16 persons suffering from epidemic meningitis, and in six found an organism resembling the meningococcus in every respect, including agglutination. Goodwin and Sholly (1906) examined the noses of

55 healthy persons not in contact with meningitis cases, and on two occasions isolated an organism which resembled the meningococcus in its cultural characteristics, but differed from it in its agglutinating properties. They also examined the noses of 45 persons in close contact with meningitis cases and found the meningococcus in five, *i.e.* 11 per cent.

v. Lingelsheim (1906) examined the naso-pharyngeal secretion of 346 persons who were in health, or ill of some complaint other than meningitis, and who were not in contact with meningitis cases: in none did he find the meningococcus. In 125 persons in more or less close contact with meningitis cases he found the meningococcus in 24 (= 19%). On the other hand, he examined the nasal secretion in 787 cases of clinical meningitis and found the meningococcus in 182 (= 25%). In 49 cases examined carefully in hospital in the first five days of their illness 46 (= 93·8%) yielded the meningococcus.

Kutscher (1906) in Berlin examined the noses of 56 patients suffering from other complaints, and two of these yielded organisms which resembled the meningococcus in every respect, including agglutination; two yielded cocci like the meningococcus, but the agglutination test was not applied as the cultures died out. According to Kolle and Wassermann (1906), 114 persons were examined in Berlin with similar results.

In the identification of *Micrococcus catarrhalis* the most important organisms that require to be considered are the meningococcus, gonococcus, and certain other races of Gram-negative cocci, which occur in the nose and elsewhere, but have not yet been clearly differentiated.

The three first-mentioned organisms resemble each other very closely in several particulars.

1. In the body the cocci are often found exclusively inside the polymorphonuclear leucocytes.

2. When first removed from the body they have very feeble powers of growing on artificial media, usually requiring the addition of blood, or ascitic fluid or serum to the medium.

3. They do not usually survive long without sub-culture.

4. They are very easily killed, especially when first isolated, by drying.

5. They are very feebly, or often not at all, pathogenic for laboratory animals.

6. Morphologically there is a tendency for the cocci to vary very much in size on artificial media, especially after a few days' growth.

Individuals then occur which are two or three times the size of the ordinary forms, stain more deeply by methylene blue, and are not so readily decolourised by Gram's method.

7. They grow in pairs or tetrads, never in chains.

8. They all, when taken from the body or from a 24 hours' culture, decolourise rapidly and completely by Gram's method, except the giant forms mentioned above, which decolourise less rapidly.

The chief recognised points of difference between the several species are the following, and here I shall make use of the description by Neisser (1903) in Kolle and Wassermann's handbook unless another author is mentioned.

1. On agar with or without ascitic fluid the colonies of *M. catarrhalis* are thicker, more raised and more opaque than those of the meningococcus or gonococcus, and they appear coarsely granular under a low power of the microscope; they readily become confluent, and are of a firm consistency.

The colonies of the meningococcus, on the other hand, are soft and sticky, and appear smooth or only finely granular with low magnification. The colonies are often confluent when crowded.

Dunham (1906) considered that the mucilaginous nature of colonies of the meningococcus and some similar organisms was useful as a means of differentiating the various groups, as he found that this property caused an emulsion of these organisms to pass very slowly through filter paper.

2. Ghon and Pfeiffer (1902) say that their cultures of *M. catarrhalis* in *broth* usually formed a skin on the surface, and later a ring at the upper level of the liquid and a deposit, the broth remaining clear, but Neisser (1903) states that the broth becomes turbid and a deposit forms. Bezançon and de Jong (1905) observed a turbidity and powdery deposit.

The meningococcus causes general turbidity, and if the tube is kept very still a surface skin is said to form; whilst the gonococcus on the addition of ascitic fluid or serum to the broth grows as a coarsely granular surface skin and deposit leaving the broth clear.

3. When media containing various carbohydrates are used for cultures, the different groups do not produce acids from the same sugars. Dunn and Gordon (1905) noted the reactions in broth containing glucose, maltose, saccharose or galactose after incubation at 37° C. for seven days. He found that *M. catarrhalis* produced acid in none of these, and that the meningococcus did so in glucose,

maltose and galactose, and the gonococcus in glucose and galactose (when a few drops of ascitic fluid had been added). F. W. Andrewes (1906) adds dextrin and laevulose to those carbohydrates from which acid is formed by the meningococcus.

Dunham (1906), using litmus-glucose-serum-broth found that the meningococcus produced acid but no coagulation, and that catarrhalis-like organisms either caused no acidity or produced acid and also caused coagulation of the medium, whereas *M. catarrhalis* caused neither acidity nor coagulation.

4. Specific agglutination as a means of differentiation has proved of value as regards several kinds of Gram-negative organisms, but *M. catarrhalis* and some of the other species agglutinate spontaneously. Dunham (1906), Goodman and Sholly (1906), v. Lingelsheim (1906), and others have prepared meningococcus sera and have successfully used their agglutinating action to separate organisms otherwise indistinguishable from the meningococcus. v. Lingelsheim also prepared a *M. catarrhalis* serum which did not agglutinate the meningococcus.

Several other kinds of Gram-negative cocci have been isolated from the nose which do not conform to the characters of *M. catarrhalis*, meningococcus or gonococcus. Some of these are said to resemble catarrhalis in forming no acid in those sugars in which they were tested, others resemble catarrhalis in most respects, but produce acid in glucose, galactose, maltose and laevulose, and also in cane sugar, as pointed out by Dunn and Gordon (1905), who found as well another race which produced acid only in glucose and maltose.

v. Lingelsheim (1906) isolated five kinds of Gram-negative cocci from the naso-pharynx and differentiated them by the size and appearance of their colonies, by the production of yellow pigment, by the changes which they produced in solid media containing various carbohydrates and coloured with litmus, and by their agglutination reactions with sera prepared by injecting the meningococcus, catarrhalis or one of two other races which he had isolated.

I have taken the following as the chief differentiating characteristics of the principal Gram-negative cocci here considered:

*M. catarrhalis*. Growth on ordinary agar after the first isolation in translucent colonies which are whitish by reflected, and brown by transmitted light, and are seen to be coarsely granular with low magnification.



Growth fairly good on gelatin at 20° C.: inability to produce acid from any of the carbohydrates on which it has been tried.

*Meningococcus.* A less free growth on agar, the colonies translucent and slightly milky by reflected light; the appearance of the colonies with low magnification smooth or only finely granular; general turbidity in broth; the production of acid from maltose and usually from glucose, galactose and laevulose, but not from cane sugar. Absence of growth at 20° C. Agglutination with a serum prepared by injecting a rabbit with the meningococcus.

*Gonococcus.* Inability to grow on media without addition of serum or ascitic fluid, at least till several generations of artificial culture have been passed. Colonies very translucent, and not confluent. Inability to form acid in maltose or saccharose although glucose or galactose may be thus affected.

I have examined the nose in 54 instances by means of small swabs, which were introduced where possible through a speculum, and then smeared on the surface of a mixture of blood and agar, or of ascitic fluid, nutrose and agar, as recommended by Dunn and Gordon. In 19 instances I isolated *M. catarrhalis*, but from a much larger proportion of infants under one year than of older children or of adults: the last furnished proportionately the fewest strains. The proportion in catarrhal noses was no higher than in the normal noses. In only one case, a girl of 16 years of age, was *M. catarrhalis* obtained in pure culture from a nose, and this was on the fourth day of a case of acute nasal catarrh without much constitutional disturbance, when the catarrh was nearly at its height.

TABLE I. *Organisms isolated from 53 noses.*

	15 Normal	7 Discharging Post S. F.	26 Acutely catarrhal	5 Meningitis cases
<i>M. catarrhalis</i> ... ..	5	3	8	3
Percentage of cases examined in which <i>M. catarrhalis</i> was isolated ... ..	33	43	31	60
Pneumococcus ... ..	4	1	9	3
Percentage of cases examined	26	14	35	60
Hoffman's bacillus	2	4	11	—
Percentage of cases examined	13	49	42	—
Catarrhalis-like organisms	1	1	1	—

TABLE II.

	Age:—	Under 1 year	1—14 years	Over 14 years	All ages
Number of noses examined	...	12	23	19	54
Number in which <i>M. catarrhalis</i> was found	... ..	7	8	4	19
Percentage with <i>M. catarrhalis</i> of those examined at each age		58	35	21	35

I examined the nose in five cases of meningitis, but not within the first five days of the illness. Many organisms appeared in the cultures, but in no instance did I find an organism resembling the meningococcus.

In order to compare the characteristics of *M. catarrhalis* with other cocci which resemble it morphologically and in their staining reactions, I have cultivated the following varieties of Gram-negative cocci.

1. Six strains of meningococcus.
2. *Strain 4M* isolated by Dr Marshall from fluid obtained by lumbar puncture in a case of meningitis supposed to be of tubercular origin.
3. *Gonococcus*, isolated by myself on blood agar from an acute case of gonorrhoea.
4. Nineteen strains of *M. catarrhalis*, which were all isolated from the nose by myself, and an additional strain, U, derived from a culture, which was very kindly furnished by Dr M. Gordon: it was obtained by him from the Hertfordshire epidemic and agreed in all particulars with my own strains.
5. *Catarrhalis-like organisms*. Of these I have isolated three strains from the nose:

*Strain Y*, from a catarrhal nose, which also yielded typical catarrhalis: this strain was very much like the type, but was constantly small and of unusually uniform size.

*Strain Q*, isolated from a normal nose, in company with the pneumococcus: it was a small coccus and its sugar reactions differed from catarrhalis.

*Strain V*, obtained in pure culture from the nose of a boy, where it was found in a purulent discharge which had been present since he had scarlet fever some months before. It resembled *M. catarrhalis* except in its sugar reactions.

I have grouped together the 19 strains which I consider to be *M. catarrhalis*, although they present minor differences as to vigour of growth and power of surviving on agar.

TABLE III.

	(Gelatin at 20° C.)	Gram.	Broth	Ascitic broth	Emulsion	Agglutination with meningococcus serum	Maltose broth	(Inucose broth	(Galactose broth	Saccharose broth	Laevulose broth	Lactose broth
Meningococcus XII	0	0	Turbid	Turbid	Good	0	A	A	A	-	A	-
" XVII	0	0	"	"	"	0	A	A	A	-	A	-
" XVIII	0 <sup>1</sup>	0	"	"	"	0	A	A	A	-	A	-
" XIX	0	0	"	"	"	0	A	A	A	-	A	-
" XXIX	0	0	"	"	"	0	A	-	-	-	A	-
" M 38	0	0	"	...	...	...	A	A	A	-	A	-
4M	+	0	"	...	Good	0	-	-	-	-	-	-
Catarrhalis 19 strains }	+	0	Clear & granules	Turbid & ring	Bad	+	-	-	-	-	-	-
" strain U	+											
Catarrhalis-like												
" Q	Fair	0	Clear sand	...	Bad	...	A	A	A	A	A	-
" V	Good	0	Clear granules	...	"	+	A	A	A	A	A	A
" Y	Good	0	Clear granules	...	"	+	-	-	-	-	-	-
Gonococcus <sup>2</sup>	0	0	...	Clear & granules	Fair	+	-	A	A	-	-	-

A = Acid production.

<sup>1</sup> XVIII did on one occasion grow on gelatin.<sup>2</sup> Ascitic fluid was added to the sugar-broth for growing the gonococcus.

As regards morphology, *M. catarrhalis* is more uniform in appearance and better stained by methylene blue than meningococcus.

The growth of *M. catarrhalis* on agar at 37° C. on first isolation was rather feeble, but after a few sub-cultures it became more vigorous; the colonies on agar were firm, so that the platinum wire in making a sub-culture broke solid pieces off or detached the whole colony, whereas the meningococcus colonies were of a slimy or honey-like consistency. On gelatin at 20° C. the growth, though often feeble at first, later became whitish and more vigorous. The gelatin was not liquefied; if the tube was incubated at 37° C. the appearance of the culture resembled that in broth, and on cooling the medium completely solidified. In ordinary *broth* cultures the medium always remained clear (unless shaken) with a coarsely granular or sand-like deposit at the bottom, usually suspended in a mucus-like ball. These cultures in broth resembled those of the gonococcus in ascitic-broth, but when ascitic fluid or sugar was added to broth and *M. catarrhalis* inoculated, growth always caused turbidity.

The catarrhalis-like organisms Y, Q, and V, from the nose, all grew in broth as a granular deposit and left the medium clear: Q forming fine sand which adhered to the glass: V forming coarse granules in a glairy ball. 4M caused uniform turbidity like meningococcus.

Carbohydrate peptone broths made with 75% peptone water, 25% peptone beef broth, and 1% of carbohydrate were used.

*M. catarrhalis* and the strains Y and 4M formed no acid in glucose, maltose, laevulose, galactose, cane sugar or lactose. Strain V formed acid in 24 hours in all these sugars, and Q in 24 hours in all of these except lactose.

Good uniform emulsions of *M. catarrhalis* and the catarrhalis-like organisms were very difficult to obtain, but the meningococcus and strain 4M were easily emulsified.

Agglutination with a specific serum as a means of differentiating *M. catarrhalis* is of little service, since all the strains cultivated by me agglutinated spontaneously, but the serum of a rabbit repeatedly inoculated intravenously with cultures of *M. catarrhalis* did not agglutinate the meningococcus or strain 4M. The gonococcus and strains Y and V all agglutinated spontaneously.

The character of spontaneous agglutination appears to be so constant as to form an important point for differentiating these groups of Gram-negative cocci, especially as the strains of meningococcus

examined by me never agglutinated spontaneously, nor can I find that other workers have observed this occurrence.

### *Conclusions.*

1. Gram-negative cocci derived from the nose can be divided into several different races, which require very careful culture for their identification.

2. *M. catarrhalis* is present very frequently in the normal nose, especially in the young and more especially in infants.

3. Its frequency does not appear to be greater in ordinary catarrhal states than in non-catarrhal. In this respect it differs from the pneumococcus and Hoffman's bacillus.

My thanks are due to Dr A. Macfadyen for having suggested the work contained in this paper and for his help and advice, and to Dr A. E. Boycott for much help and advice throughout.

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## A NOTE ON THE INFLUENCE OF THE CHEMICAL RAYS OF DAYLIGHT ON VACCINIA IN ANIMALS.

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IN view of the observations made of late years on the action of red light in variola, it was thought that it might be of interest to ascertain, if possible, whether the red, or other rays of daylight, exerted any influence in cases of vaccinia; that is to say, with a disease the specific virus of which differs from that of variola probably only in that its pathogenic capabilities are modified, while vaccinia presents clinically many points of resemblance to variola.

As it was conceivable that any influence which rays of daylight might exert *in vivo* might also be exhibited on the virus outside the animal body, in the first instance experiments were made on vaccine material *in vitro*.

### *Experiments on vaccine emulsion in vitro.*

Vaccine emulsions were subjected to the influence of variously coloured rays of daylight in the following manner:—

Vaccine pulp was collected in the usual way from a calf 120 hours after the animal had been vaccinated. A portion of this pulp was emulsified with four times its own weight of sterile distilled water, and was subjected to the chloroform process. A second portion of the pulp was emulsified with four times its own weight of a 50% mixture of pure glycerine and sterile distilled water.

The chloroformed emulsion was partly used to fill seven small glass vessels of test tube shape each having a capacity of 5 c.c. Each tube was filled with vaccine and tightly corked.

One of each of these seven tubes was placed in a separate glass compartment. The first compartment was made of red glass, the second of yellow, the third of green, the fourth of blue, the fifth of violet, the sixth not coloured, and the seventh, also of uncoloured glass, was itself enclosed in a light-tight tin box.

The remainder of the chloroformed vaccine emulsion was put into *glass capillary tubes* and six of these filled capillary tubes were also placed in each of the seven above mentioned glass compartments. Capillary tubes were used in order that a thin column of vaccine emulsion, as well as the thicker columns of vaccine in the small test tubes, might be subjected to the various coloured rays.

The glycerinated emulsion was placed in small test tubes and glass capillary tubes in a similar manner and these were similarly placed in the compartments.

The compartments themselves were placed in a laboratory window facing north and were all equally subjected to the action of daylight throughout the day. Altogether vaccine from seven calves was treated in this manner, the exposure in the glass compartments lasting continually from the middle of July to the end of February. At the end of this time some rabbits and two calves were inoculated with all the seven vaccines of each compartment, a sample of each vaccine being taken from both a capillary tube and from one of the small test tubes.

It is needless to tabulate the result of these inoculations, for the vesiculations of both calves and rabbits failed to show any difference between the various portions of any one of the seven vaccines. Possibly the vaccine from the small test tubes gave rather better vaccination results than did the vaccine from the capillary tubes, but further than this no difference could be noted.

It would appear from these *in vitro* experiments that the resistance of the specific virus of vaccinia to the germicidal action of daylight is greater than the resistance usually shown by non-spore-bearing micro-organisms, and approximates more closely to the resistance of spore-bearing bacteria.

#### *Experiments on Vaccinated Animals.*

The animal experiments were conducted as follows:—Three rabbits, young animals—six to twelve weeks old—and as similar as possible in size and condition, were taken. Each animal had an area, roughly  $4 \times 5$  millimetres, shaved on its back. The areas of each set of three

rabbits were inoculated in linear incisions with vaccine lymph from the same calf.

Immediately after inoculation the first rabbit was placed in a cage which had been closely covered with a double layer of red glazed cambric such as is used for photographic dark rooms. This covered cage was so situated that it received on its top and four sides direct daylight from sunrise to sunset. The red cover of the cage was only partially and momentarily removed twice in the 24 hours during the first 72 hours after the inoculation of the rabbit, for the purpose of placing the food inside the cage. After the first 72 hours the rabbit was removed for a few minutes to examine the condition of its vaccinated area, after which it was returned to the red light for a further 24 or 48 hours.

The second rabbit of the batch was, immediately after inoculation, placed in the dark, light being admitted to the cage momentarily only when feeding the animal. The rabbit was kept thus for 72 hours after inoculation, at the end of which time—as in the case of rabbit No. 1—it was removed for a few minutes to ascertain the condition of its vaccinated area. After examination it was replaced in the dark for 24 or 48 hours or more.

In experiments with red light in small-pox<sup>1</sup> any advantage which may have accrued in consequence of “red light treatment” may obviously have been due to the red light *qua* red light or to the fact that the chemical rays were occluded. By using a dark cage as an adjunct to the red light the advantage of the latter should be emphasised or detracted from as the case might be.

The third vaccinated rabbit was kept in such a way that it was in direct daylight during the daylight hours of the first 120 hours or more after its inoculation.

The cages of all three rabbits were arranged close together so that apart from the admission or occlusion of light rays, the animals were under the same conditions. The majority of the experiments were made at a time of year when it was daylight during more than 12 of the 24 hours. 31 sets of rabbits were thus treated (93 animals in all).

The results of these vaccinations were noted at varying intervals and are set forth in the accompanying Table.

The various degrees of development have been classified, somewhat

<sup>1</sup> *vide* Finsen, *Brit. Med. Journ.*, 1903, Vol. I. p. 1297, and *Lancet*, 1904, Vol. II. p. 1272. Schamberg, *Journ. Amer. Med. Assoc.*, 1903, Vol. XL. p. 1183, and 1904, Vol. XLIII. p. 1641. Ricketts and Byles, *Lancet*, 1904, Vol. II. p. 287, and 1904, Vol. II. p. 816.

roughly, into four classes. Class 1 includes all vesicles containing lymph of high virulence, Class 2 all vesicles containing lymph of secondary virulence, Class 3 vesicles containing lymph of virulence next below that of 2, and Class 4 all vesicles containing lymph of low virulence. Absence of vesiculation is recorded as 0. This classification takes no note of any sign other than the appearance of the vesicle itself.

The appearance of each areola was recorded and, according to its degree of intensity, was noted under one of three headings, the most extensive being 1, the medium 2, and that below the medium 3, while absence of areola was noted as 0.

TABLE.

Nature of Exp.	Class of areolae				Class of vesicles				
	1	2	3	0	1	2	3	4	0
Red light	8	8	8	7	23	1	2	4	1
Dark	11	14	4	2	17	1	5	8	0
Daylight	10	12	6	3	15	2	4	10	0

Dealing in the first instance with the tabulated results of the areolae, it will be observed that the largest number of the most severe areolae occurred in those animals protected from all light rays. Those animals which were exposed to red light had the smallest number of the severest type of areola, while the "daylights" had an intermediate number. Again, in the case of those animals which had least extensive areolae, if the "red lights" and the "daylights" are examined it will be seen that the former have the greatest numbers, whereas those animals protected from all light rays had a smaller number of mild areolae than had the "daylights." The same contradiction of results is seen if one examines the totals of those animals which showed no areolae, for of these the "red lights" are in the greatest number, and the "darks" in the least, the "daylights" being intermediate.

It is clear that in the case of these experiments no deduction can be drawn as to the action of any of the rays of daylight in the production of vaccination areolae, unless it be that the degree of areola is uninfluenced by the presence or absence of daylight.

Proceeding to the examination of the vesicles it will be seen that by taking the actual total numbers of the results, it would appear that a distinctly larger number of cases exposed to red light developed vesicles of a highly virulent type than did those cases exposed to daylight; while those cases left in the dark, and therefore also excluded from the chemical rays, gave a somewhat larger number of virulent vesicles than did the cases exposed to daylight.



These figures are corroborated by examining the numbers of vesicles of low virulence developed in red light, dark, and daylight respectively. It will be seen that the proportion of vesicles of low virulence was rather larger among the "daylight" than among the "dark" animals, and much larger than among the "red lights."

In order to gain additional information on this point three goats were vaccinated and treated in a manner similar to that adopted in the case of the rabbits. The goats were placed in red light, daylight, and dark respectively, and the results of the vaccinations were noted from the sixth to the tenth days. In respect to vesiculation the results were in complete accordance with the results of the experiments with rabbits, for the goat exposed to red light developed vesicles of first-rate quality, the goat left in the dark developed vesicles almost as good, while the goat left in daylight yielded only poor vesicles.

As a further experiment four more goats were vaccinated and treated in a manner similar to that of the former three, except that it was not found practicable to use red light. One of the animals was placed in the dark, and the other three were tethered during the course of the experiment in an unshaded field. This experiment was carried out in the middle of June, 1906.

On the sixth, eighth, and tenth days after vaccination the goat excluded from daylight showed exceptionally good vesicular development, while none of the three animals kept in daylight showed any appearance of real vesiculation, dried lines of crust only appearing along the lines of inoculation.

A few days later a further experiment was made with two calves. Each of these calves was vaccinated from the same batch of vaccine lymph, following the procedure of the former experiments. One of the calves was placed in the dark, while the other was kept in the open field.

On the fifth day after inoculation the calf protected from daylight showed well-developed, typical vesicles, while the calf kept in the field failed to show more than the merest trace of vesiculation. This second animal was kept under observation under the same conditions for three weeks, during which time no further vesiculation appeared.

From these experiments it would seem at least to be strongly indicated:—

(1) That chloroform water emulsions, and glycerine water emulsions of vaccine lymph *in vitro* are not appreciably affected with regard to their potency by exposure to or protection from daylight. The vaccine

virus indeed would appear to resemble in this respect ordinary bacterial spores rather than the usual non-spore-bearing bacteria.

(2) The development of the areola in vaccinated rabbits is apparently unaffected by the exposure of these animals to the chemical rays of daylight, or by protecting them from such rays.

(3) That vaccinia in rabbits, goats, and probably in calves, *as a specific disease* is influenced in such a way by the prolonged exposure of the vaccinated animals to the chemical rays of daylight that its development is prevented to a greater or lesser extent.

Should this last point be established, the advantage in a vaccine establishment of protecting the animals used for the production of vaccine lymph from the rays of daylight is obvious.

## PUBLICATIONS RECEIVED.

## BOOKS.

BARDSWELL, N. D. (1906.) *The Consumptive Working Man; What can Sanatoria do for Him?* London: The Scientific Press Limited, 28 & 29, Southampton Street, Strand, W.C. 202 pp., 4 plates. 22 × 14 cm. Price 10/6 net. Cloth.

The author, under this somewhat general title, carefully describes the progress of twenty-five working men in relation to sanatorium treatment. The cases are well chosen from a large number observed in the course of several years and they bring out the influence of social and economic conditions upon the course of the cases.

BULLOCH, W., (*Editor*). (1906.) *Studies in Pathology* written by Alumni to celebrate the quarter-centenary of the University of Aberdeen and the quarter-centenary of the chair of pathology therein. 412 pp. 26 × 19 cm. Cloth. Printed by Milne and Hutchison, Aberdeen.

This work contains the following original papers :—The Alimentary Canal as a Source of Contagion, by Prof. D. J. Hamilton, pp. 1–38.—A Remarkable Case of Bilharziosis, by W. St Clair Symmers, 8 figs., pp. 41–54.—Malformations of the Bulbus Cordis, by A. Keith, 10 figs., pp. 55–74.—The Administrative Aspects of Tuberculosis, by W. L. Mackenzie, pp. 77–94.—Paroxysmal Irregularity of the Heart and Auricular Fibrillation, by A. R. Cushny and C. W. Edmunds, 6 figs., pp. 97–110.—Researches on certain Problems of Plague Immunity, by G. Dean, pp. 113–155.—Experimental Study of the Immunity against *Bacillus Pyocyaneus*, by W. Bulloch, pp. 159–174.—On *Epignathus*, by A. Low, 6 figs., pp. 177–198.—A Contribution to the Pathology of Exophthalmic Goitre, by G. M. Duncan, 6 figs., pp. 201–214.—The Rat Theory of Plague Epidemics, by W. Hunter, Charts I–VII, pp. 217–236.—Some Experiments with Disinfectants, by A. R. Laing, pp. 239–263.—On Eck's Fistula—observations on Four Dogs, with a review of the literature relating to previous work on the subject, by J. J. R. Macleod, pp. 267–286.—On the Action of certain Bacteria in producing Cell-necrosis, with Special Reference to those of the *Bacillus Enteritidis* (Gaertner) Group, by G. F. Petrie, pp. 289–298.—The Relationship between the Factors inducing Haemolysis and those including the Phagocytosis of Red Blood Corpuseles, by R. D. Keith, pp. 301–319.—An Experimental Enquiry into the Relationship of Leucocytosis to the Opsonic Content of the Blood Serum, by J. C. G. Ledingham and W. Bulloch, Charts I–XXXI, pp. 323–364.—Immunity in Pneumococcal Infections, by G. G. Macdonald, 3 Charts, pp. 367–399.—Preliminary Note on the Bacteriology of some Diseases of Sheep, by J. M. Adams, and B. R. G. Russell, pp. 403–410.

- CALMETTE, Dr. (1907.) *Recherches expérimentales sur la Tuberculose effectuées à l'Institut Pasteur de Lille.* Paris: Masson et Cie., Editeurs. Fasc. I. (1906.) 131 pp. 22 × 16 cm.

The fasciculus summarizes the results of the author's very important investigations on the pathogenesis of tuberculosis and on the defence of the body against invasion by the tubercle bacillus. These results have already been published in collaboration with Messrs C. Guérin, P. Vansteenberghe, M. Breton, Grysez, Sonnevile and G. Pétit in the form of papers read before various scientific societies. Nevertheless, in view of their importance, the collection of these scattered papers into a single volume will prove most welcome to those interested in the progress of the scientific and experimental investigation of tuberculosis.

- EHRlich, P. (1906.) *Collected Studies on Immunity.* (Translated by Dr Charles Bolkuan of New York.) New York: John Wiley & Sons, 43 E. 19th St.; London: Chapman & Hall, Limited. 586 pp. 23 × 16 cm. Cloth. Price, \$6.00.

For those who are unable to read German fluently, an English translation of the classical papers incorporated in this work must necessarily prove most welcome. Important investigations published since the appearance of the work in German are incorporated in the translation and bring the subject up to about March 1906. The translator deserves full recognition for his successful and painstaking efforts.

- GALLI-VALERIO, B., and ROCHAZ-DE JONGH, J. (1906.) *Manuel pour la Lutte contre les Moustiques.* Lausanne: Librairie Nouvelle. Paris: A. Maloine, Éditeur. 245 pp. 94 figs. 16 × 10 cm. Cloth. Price Fres. 4.50.

This little manual gives a short description (four chapters) of the structure, biology, classification and pathogenic rôle of mosquitoes. Chapter V deals with the technique of investigations on mosquitoes and the diseases they convey. Chapter VI deals with the methods of mosquito destruction, and a section, entitled General Conclusions, completes the volume. Many of the authors' own investigations, in part already published, are incorporated in the book which is well worthy of notice at the hands of those who have to deal with the matters of which it treats.

- HAMER, W. H. (1906.) *The Milroy Lectures on Epidemic Disease in England.—The Evidence of Variability and of Persistency of Type.* (Delivered before the Royal College of Physicians of London, March, 1, 6 and 8, 1906.) London: Printed at the Bedford Press, 20 & 21, Bedfordbury, London, W.C. 72 pp., 1 Table. 22 × 14 cm. Cloth.

- KOLLE, W., and HETSCH, H. (1906.) *Die Experimentelle Bakteriologie und die Infektionskrankheiten mit besonderer Berücksichtigung der Immunitätslehre.* Urban & Schwarzenberg, Berlin: N., Friedrichstrasse 105 b. Wien: I., Maximilianstrasse 4. 589 pp., 3 plates, 125 mostly coloured figs. 25 17 cm. Price M. 20. Cloth.

The above work is intended for students, physicians and medical officers of health. It gives in condensed form a review of our present knowledge of bacteriology and infective diseases, leaving controversial matter out of account. The material is presented in the form of lectures, 53 in number, so as to render the perusal of the work easier than in most text-books. The main object of

the authors has been to bring out clearly the relations existing between experimental bacteriology and the study of the epidemiology, diagnosis, prophylaxis and cure of infective diseases. Reproductions of coloured drawings illustrate the text, many of these are given in place of the usual photomicrographs which at times confuse the beginner. Twelve chapters are devoted to Bacteria, Infection and Immunity, the remainder to the discussion of the different infective diseases, including five chapters dealing with the Protozoa. The work presents a very attractive appearance and should prove most useful to those for whom it is intended. It is fully abreast of the times, which is more than can be said of many works dealing with the subject.

MOOR, C. G., and HEWLETT, R. T. (1906.) *Applied Bacteriology*. An Elementary Handbook for the use of Students of Hygiene, Medical Officers of Health and Analysts. 3rd ed. (University Series). London: Baillière, Tindall & Cox. x+492 pp., 29 plain and 81 coloured illustrations. 22 × 15 cm. Cloth. Price 12/6 net.

That this elementary handbook has proved useful to many workers is proved by its appearance in third edition. It can be thoroughly recommended, especially to beginners.

PORTER, C. (1906.) *School Hygiene and the Laws of Health*. Longmans, Green & Co.: 39, Paternoster Row, London. 313 pp., 119 illustrations in text. 19 × 13 cm. Price 3/6. Cloth.

This book is based on lectures delivered to teachers and students of the Training College, Sheffield. The lectures were arranged for students preparing for the Sanitary Institute examination in School Hygiene. Whilst preserving an elementary character the book contains a good deal of information in condensed form.

ROBERTS, E. (1906.) *Enteric Fever in India and in other tropical and sub-tropical regions. A Study in Epidemiology and Military Hygiene*. London: Baillière, Tindall & Cox, 8, Henrietta St., Covent Garden. 571 pp., 36 Maps, Charts, and Diagrams. 27 × 18 cm. Price 21/- net. Cloth.

The author states, that whilst typhoid fever provides the text, his whole purpose has been to present in the foregoing volume, a study of the main problems of epidemiology and of military hygiene. The work discusses the etiology and epidemiology of typhoid fever and is divided into ten chapters:

I. Introductory. Historical Survey of the influences which have affected the character of the personnel of the British Army in India and of the diseases to which it was subject during the last half of the 19th Century. II. Course and Rise of Enteric Fever among the European troops serving in India. The validity of the record and estimate of the true extent of the prevalence of Enteric Fever, with an analysis of the returns on account of other Fevers and bowel diseases. Influence of changes in the personnel on the course of Enteric Fever prevalence, and of age and service (including previous experience) on the chief causes of sickness and mortality. Practical indications. III. Etiology with special reference to the terms of the epidemiological problem in India; the human host, the specific agent of infection, the environment. Paratyphoid infection. IV—VI. Epidemiology. (1) Local and Seasonal incidence of the disease in India and elsewhere, with a discussion of the determining factors. (2) Evidence



of Importation. Camp and Campaign influence; historical survey and summary. (3) Incidence on the different arms of the Service; on Corps units; on Officers, Women and Children. VII. Summary of the Epidemiology; sources of infection. VIII and IX. Enteric Fever among Natives of India and other tropical and subtropical regions. Discussion of the biological factors in operation as affecting the question of a relative immunity possessed by Natives. Analysis of the evidence. Geographical survey of the distribution of the disease from original sources of information. X. Conclusions and Indications. Prophylaxis and Suppression.

SAVAGE, W. G. (1906.) *The Bacteriological Examination of Water-Supplies*. London: H. K. Lewis, 136, Gower Street, W.C. 297 pp., 13 figs. 21 × 13 cm. Price 6/6 net. Cloth.

The author has had a considerable experience in the bacteriological examination of water. The book will commend itself to those engaged in such work. A good bibliography of recent work on the subject will be found at the end of the volume. Part I deals with the Influences affecting bacteria in water—quantitative bacterial content of natural waters—bacteriology of excreta, sewage and soil in relation to bacteriological examination of water—*Bacillus coli* and allied organisms—the Eberth or typhoid group—other intestinal bacteria—the content of various waters in *B. coli*, *B. enteritidis sporogenes*, *streptococci*—bacterial indicators of pollution—interpretation of results in the bacteriological examination of water—Classification of bacteria found in water. Part II. Collection and transmission of samples—general quantitative examination—methods of enumerating and identifying *B. coli* and allied organisms—examination of water for the typhoid bacillus and for other intestinal organisms. Appendix relating to methods.

THRESH, J. C., and PORTER, A. E. (1906.) *Preservatives in Food and Food Examination*. London: J. & A. Churchill, 7, Great Marlborough Street. 484 pp., Pls. I–VIII. 24 × 16 cm. Price 14/- net. Cloth.

In view of the greatly increased use of preserved food, especially of recent years, a work dealing with the subject in the light of recent knowledge must necessarily be a welcome addition to the literature. The work falls into five parts: I. Chemical preservatives and their physiological effects. II. Milk and milk products; alcoholic and other beverages; fruits, jams and vegetables; meat, game, eggs and fish. III. Colouring matters and mineral poisons. IV. Food inspection laws; unsound food, meat, public abattoirs, veterinary inspection, animal diseases, etc.; animal parasites; fish, shellfish and bacteriological examination thereof; Milk and milk products, and vegetables in relation to disease, etc.; food poisoning. V. Detection and estimation of preservatives and metallic impurities, examination for coal-tar colours, legal cases. Abstracts from the Departmental Committee's Report and the Law of practice in Foreign Countries and the Colonies as to preservatives, etc. conclude the volume in the form of two appendices.

VERNON-HARCOURT, L. F. (1907.) *Sanitary Engineering with respect to Water-supply and Sewage Disposal*. (Civil Engineering Series.) Longmans, Green & Co., 39, Paternoster Row, London. 419 pp., 287 Illustrations. 23 × 16 cm. Price 14/- net. Cloth.

This work comprises sixteen chapters dealing with: (Part I. Water-Supply.) Ancient waterworks; available rainfall—Sources of water supply—Wells—Deep Wells—Lakes and Storage Reservoirs—Earthen and Rubble Reservoirs—Dams—Masonry Dams—Typical Masonry Dams—Intakes, and conveyance and storage of supply—Purification of water-supplies—Distribution of water-supply. (Part II. Sewage Disposal.) House drainage, and disposal of refuse—Sewerage—Outfalls; and clarification of Sewage—Utilization and purification of sewage on land—Chemical, electrolytic, and bacterial purification of sewage.

The book can be thoroughly recommended as a scientific and practical work.

## BROCHURES AND DISSERTATIONS.

- BAIJNATH, RAI BAHADUR LALA. (1906.) *The Plague in India*. Its Causes, Prevention and Cure compiled from various authoritative sources: English and Indian with the assistance of Surgeon-Major V. D. Vasu, I.M.S. Meerut: The Vaishya Hitkari Office. 64 pp. Price 4 Annas. Paper.
- D'HEIL, R. (1906.) *Beitrag zur Frage des Bakteriengehalts der Milch und des Euters*. (Inaug. Dissert. Giessen, Dr. veter. med.) *Arb. a. d. Hyg. Inst. der Kgl. Tierärztlichen Hochschule zu Berlin*, No. VII. 49 pp.
- HEISRATH, F. (1904.) *Über die Behandlung der granulösen Augenentzündung mit besonderer Berücksichtigung des Operationsverfahrens*. Leipzig: Verlag von Johann Ambrosius Barth. 46 pp. Price M. 0.80. Paper.
- KLEIN, E. (1906.) *Ueber das Vorkommen von Schweineseuchebakterien und diesen ähnlichen Bakterien in der Nasenhöhle des Schweines*. (Inaug. Dissert. Giessen, Dr. veter. med.) *Arb. a. d. Hyg. Inst. d. Kgl. Tierärztlichen Hochschule zu Berlin*, No. X. 32 pp.
- MAGELSEN, A. (1906.) *Norway as a winter and summer health resort*. Kristiania: Printed by Nikolai Olsen. 48 pp., with a number of plates and figures.
- STRUBEN, E. D. (1906.) *Over de verlichting bij het huiswerk van Schoolkinderen*. (M.D. Thesis, Amsterdam.) Venlo: Typ. Wed. H. H. Uyttenbroeck. 120 pp. 23 x 15 cm.

## NEW JOURNALS.

*Archives de L'Institut Pasteur de Tunis*. (Protectorat Français—Gouvernement Tunisien. Direction de l'Agriculture et du Commerce.) Fasc. 1-4. (Jan.-Oct. 1906.) 184 pp. Numerous Charts and plates. Tunis: Imprimerie Moderne (J. Orliac), 14 rue d'Autriche.

The object of these new archives is to afford a vehicle for the publication of papers from the Tunis Pasteur Institute, and to review publications dealing with disease in Tunis. The Fasciculi contain: I. Description of the Pasteur Institute, Tunis. II. Résumé of the work done in the Institute, its administration, bibliography of papers dealing with infective diseases in Tunis. III. C. Nicolle and Cathoire [pp. 97-132] Étude d'une épidémie de fièvre typhoïde africaine. Existence en Tunisie des infections paratyphiques. IV. Ed. Sergent and Et. Sergent [pp. 137-141] Campagnes anti-paludiques

en Tunisie. Nicolle and Cathoire [pp. 142-154]. Sur une épidémie de dysenterie bacillaire africaine. Nicolle [pp. 155-158]. Une observation de fièvre méditerranéenne par contamination de laboratoire. Nicolle [pp. 159-170]. Recherches expérimentales sur la lèpre. Catouillard [pp. 171-183]. Études de quelques levures sélectionnées employées pour la vinification.

*Archives de l'Institut Royal de Bactériologie Camara Pestana*, Lisbonne. Tome I. Fascicule 1 (Mai, 1906), 194 pp. Published by the Institute, Lisbon. 25 × 17 cm.

This new Journal contains in its opening number:—Sur la méningite cérébro-spinale épidémique et son agent spécifique (Planches I-II), by A. Bettencourt and C. França.—De l'action de quelques agents chimiques et physiques sur le Bacille de la peste, by A. Fernando Rocha.—Sur la formule hémolencocytaire de la lèpre, by C. de Lima.—Sur un Trypanosome du Blaireau (*Meles taxus* Schr.) (Planche III), by A. Bettencourt and C. França.—Contribution à l'étude des agréssines (1<sup>er</sup> mémoire), by N. Bettencourt.—Études sur la rage dans la série animale. I. La rage chez les Muridae (Murinae et Microtinae), by C. França.—Recherches sur les Trypanosomes des Amphibiens. I. Les Trypanosomes de la *Rana exculenta* (Planches III et IV), by C. França and M. Athias.—Note sur l'existence du *Trypanosoma cuniculi* en Portugal, by A. Bettencourt and C. França.—Sur le traitement des Rats infectés par le *Trypanosoma gambiense* au moyen de l'acide arsénieux et du trypanroth, by A. de Magalhães.—Le traitement antirabique à l'Institut royal de bactériologie Camara Pestana en 1905, by M. Athias.—Sur un Trypanosome de la Chauve-souris, by A. Bettencourt and C. França.—Sur les infiltrations périvasculaires de la rage (Note préliminaire), by C. França.

*Hygienisches Centralblatt*. Vollständiges internationales Sammelorgan für das gesamte Gebiet der Hygiene. Edited by Dr Paul Sommerfeld. Vol. I. No. 1 (March, 1906). 32 pp. Leipzig: Verlag von Gebrüder Borntraeger. New York: G. E. Stechert & Co. London: Williams & Norgate. Paris: Albert Schulz. 25 × 16 cm. Price 30/- a volume.

This new journal will appear twice a month, twenty-four numbers composing the volume. It is modelled on the lines of the *Biochemisches Centralblatt* and will contain early reviews of original papers, and collective reviews by specialists in the various departments. Authors' reviews are especially requested. These, as well as publications for review should be addressed to the gentlemen who have taken charge of the reviews of publications appearing in each country. Publications etc. appearing in Great Britain and the Colonies should be sent to Dr F. C. Lewis, Examination Hall, M.A.B., Victoria Embankment, London, W.C. Publications appearing in the United States should be forwarded to Prof. Burton Opitz, Columbia University, 437 W. 59th St., New York. The names of gentlemen representing the journal in other countries are given on the cover of the *Centralblatt*.

*Internationales Centralblatt für die gesamte Tuberkulose-Literatur*. Unter Mitwirkung zahlreicher Fachgelehrten des In- und Auslandes. Editors: L. Brauer, Oskar de la Camp, G. Schröder. Würzburg: A. Stuber. Jahrg. I, Nos. 1-5, pp. 1-120. Published monthly. Subscription M. 8 a year. 25 × 17 cm.

This new monthly international journal is intended to serve the purpose of

collecting the very scattered literature on tuberculosis in the form of reviews of original contributions and books dealing with the subject. It will also include reports from various congresses and societies. The editors have secured a large body of collaborators to undertake the arduous task of reviewing. The five first numbers contain 70, 32, 37 and 26 reviews respectively, not including book reviews. The *Centralblatt* will necessarily appeal to a very large number of readers and the editors are to be congratulated upon their useful undertaking.

*La Bulgarie Médicale.* Revue Mensuelle. Edited by Chr. Doctoroff, Vol. I, Nos. 4-10 (April-Oct. 1906), pp. 49-160. Sofia: Imprimerie de la Cour. (See Notice in *Journ. of Hygiene*, vol. VI, p. 100.)

The original papers contained therein are as follows: Stoianoff, P., Les rayons de Röntgen à la portée de tous les médecins.—Béron, B., La lèpre en Bulgarie.—Petroff, A. M., Une modification du procédé d'Ollier pour l'opération des épithéliomes étendus à toute la lèvre inférieure.—Buraïs and Finck, Electrothérapie pratique.—Baïtcheff, Le traitement antirabique à l'Institut bactériologique de Sofia pendant les années 1902-04.—Doctoroff, Sur l'importance de la parasitologie et son état chez nous (in Bulgaria).

*Revista Stiintelor Medicale.* Edited by Prof. Dr I. Cantacuzino, Vol. I, Nos. 2-3. (II-III, 1906), pp. 165-431. Bucaresti: Institutul de Arte Grafice "Carol Göbl." S-sor Ion St Rasidescu, 16, Strada Doamnei. 25 × 17 cm.

The above numbers of the *Revista* contain the following papers: Riegler, E., Pentozuria.—Niculesca, D. D., Fistulale cutanate de origină dentară.—Parhon and Nadejde, Gr., Recherches sur l'origine du facial supérieur chez l'homme.—Marie, A., Turbarea experimentală.—Felix, F., Turburările laringotracheale provocate de aneurisme aortei.—Friedman, T., Contribution à l'étude de la séroration agglutinante dans la tuberculose, sa valeur comme moyen de diagnostic.—Mamulea, T., and Marbe, S., Notă asupra diagnosticului tific al lui Fieker. Besides these papers the numbers devote space to short reports on clinical and pathological anatomical cases, notices of meetings of societies, reviews, etc.

## CURRENT JOURNALS, ETC.

*Atti della Società per gli Studi della Malaria.* Vol. VII. 1906. 5 plates and many figs. Roma: Società per gli Studi della Malaria. 691 pp. 25 × 18 cm.

Contains 45 papers dealing with malaria.

*The George Washington University Bulletin.* Department of Medicine Number. Vol. V, No. 3. Published by the University at Washington, D.C. October, 1906. 102 pp. 23 × 15 cm.

Contains papers chiefly on clinical subjects.

## REPORTS.

*Annual Report 1905, Shanghai Municipal Council.* Health Department. By A. Stanley (1906). Shanghai: Printed by Kelly & Walsh, Limited, Nanking Road. 57 pp. 24 × 19 cm. Boards.



- ELKINGTON, J. S. C. (1906.) *Annual Report of the Department of Public Health for the year 1905-6*. Tasmania: John Vail, Government Printer, Hobart. No. 14, 1 Table, Appendix I-IV, 13 pp.
- Annual Report of the Medical Officer of Health, and of the General Medical Superintendent of the City Hospitals*. Bristol: Bennett Brothers, Ltd., Printers, Counterslip. 141 pp., 5 Tables. 24 × 16 cm.
- Annual Report (1906) of the Medical Officers of Health, and of the Chief Port Inspector of Nuisances for the year 1905, including Report on Canal Boat Inspection*. (Printed by order of the Port Sanitary Committee.) Bristol: Bennett Brothers, Ltd., Printers, Counterslip. 35 pp. 25 × 16 cm.
- Annual Report of the Medical Officer of Health for Hertfordshire for the year 1905*. Prepared by direction of the County Council for the County of Hertford by F. E. Freemantle (1906). 128 pp., 42 Tables, 1 Map. 24 × 16 cm.
- Biennial Report of the Department of Health of the City of Chicago for the years 1904-1905*. By Chas. J. Whalen, Commissioner of Health. 368 pp., numerous diagrams and plates. 22 × 14 cm.
- Communications Statistiques publiées par le Bureau municipal de Statistique d'Amsterdam*. 1906. No. 16. Statistiek der bevolking van Amsterdam en eenige voorname steden der wereld in de Jaren 1899-1905. (Tableaux de Statistique démographique d'Amsterdam et de quelques grandes villes du monde dans les années 1899-1905.) Amsterdam: Johannes Müller. 57 pp. Price f. 0.30.
- HILL, E. (1906.) *Report of the Health Officer for the year ended 31st December, 1905*. Pietermaritzburg: "Times" Printing and Publishing Company, Limited. 39 pp., 3 Charts. 33 × 21 cm. Price 3/3.
- Report on Epidemic Cerebro-Spinal Meningitis in India*. (Issued under the authority of the Government of India by the Sanitary Commissioner with the Government of India, Simla.) By C. J. Robertson-Milne (1906). Calcutta: Office of the Superintendent of Government Printing, India. 67 pp., 7 Charts. 25 × 17 cm. Price Re. 1 or 1/6. Boards.
- Report on the Health of the City of Manchester, 1905*, by J. Niven (1906). Manchester: Henry Blacklock & Co. Ltd., Printers, Albert Square. 356 pp. 25 × 15 cm.
- Report on the treatment of Plague with Yersin-Roux serum at the Maratha Hospital during 1905*. By Khan Bahadur N. H. Choksy (1906). Bombay: Printed at the Akhbar-I-Sondagar Press. 20 pp.
- Results of a Census of the Transvaal Colony and Swaziland taken on the night of Sunday the 17th April, 1904*. (Presented to his Excellency the Governor, May, 1906.) London: Printed by Waterlow & Sons, London Wall. 755 pp. 39 × 33 cm. Boards.
- Second Annual Report of the Henry Phipps Institute for the Study, Treatment, and Prevention of Tuberculosis*. February 1, 1904, to February 1, 1905. Published by the Henry Phipps Institute, 238, Pine Street, Philadelphia. 25 × 17 cm.
- The volume contains: The Work of the Year, by Laurence F. Flick, pp. 4-49.—Autopsy Report, by C. Y. White, pp. 50-86.—Laryngological Work, by W. G. B. Harland, pp. 87-92.—Neurological Work, by D. J. McCarthy, pp. 93-136.—The Mental attitude in Tuberculosis, by H. Carnecross, pp. 137-146.—Dermatological Report, by J. Frank Wallis, pp. 147-150.—The Kidneys



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ON METEOROLOGICAL FACTORS IN THE  
AETIOLOGY OF ACUTE RHEUMATISM.

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MANY writers having observed that cases of acute rheumatism are not distributed evenly throughout the year, much study has been devoted to the possible relationship between these variations and meteorological conditions.

On this question difference of opinion exists. The problem is a complex one since any complete investigation must include the study of several factors the intensity of which is variable. One of these, humidity, has attracted particular attention, being regarded by many as a causative agent (Garrod<sup>1</sup>, Lebert, etc.). On the other hand, some authors consider the relation between rheumatism and humidity to be an inverse one (Longstaff, Newsholme, etc.). Gabbett (1883) could trace no marked coincidence between the curves of rheumatism and rainfall in his series, and Přibram (1899, pp. 355—356) found that "Dagegen war eine auffallende Beziehung zwischen dem Gange der Curve der Niederschläge und der Feuchtigkeitsprocente der Luft und dem Auftreten des acuten Gelenkrheumatismus (im Jahresdurchschnitte) insofern zu erkennen, als im allgemeinen der mehrjährigen Dauer rheumatismusarmer Zeiten, reichlichere Niederschläge und grössere Feuchtigkeit, dagegen den mehrjährigen rheumatismus-reichen Epochen im allgemeinen geringere Niederschlagsmengen und geringere Luftfeuchtigkeit zu entsprechen schienen." In the hope of throwing some light on this question we have recently analysed the statistics of acute

<sup>1</sup> For a full account of the literature see Přibram (1899).

## 172 *Meteorological Factors and Acute Rheumatism*

rheumatism admissions to the London Hospital, between the years 1873—1903, and we shall describe in this paper the results obtained.

Before going into the details of this work, it is important to consider certain matters bearing on the general nature of hospital statistics with special reference to the problem under discussion. In a memoir dealing with the statistical results obtainable from the post mortem rooms of a general hospital, one of us (Greenwood, 1904) pointed out that a "General Hospital Population" differs very materially not only from a random sample of the general population, but from one of diseased persons who do not seek hospital treatment. Not only are the majority of hospital inmates drawn from a particular class of the community, but, further, there is a well marked tendency for certain groups of disease to be over-represented.

"Evidently the population of a general hospital will chiefly consist of (1) persons acutely ill, (2) those suffering from surgical injuries or diseases, (3) sufferers from medical affections requiring special treatment. Chronic maladies of old age, such as bronchitis, indeed, any highly chronic disease, will be under-represented in comparison with the general death-rate. Similarly the number of cases of valvular heart disease and rarer disorders, such as diabetes mellitus or insular sclerosis and other nervous lesions, will be above the general average," (Greenwood, 1904, p. 65).

If these considerations be sound, the actual or relative frequency of, for example, acute rheumatism admissions cannot serve as a basis for general reasoning regarding the incidence of this disease among the ordinary population. But a further deduction must be made. Since certain diseases are over-represented in the "General Hospital Population," it must follow that a system of preferential admissions exists, certain types of affection being excluded. If acute rheumatism be among this number, the fact that during certain periods, more cases of acute rheumatism were admitted than usual, would only mean that some other disease was less prevalent, and we could not infer that the causative factors of the former malady were especially active.

We have carefully investigated this point, and our conclusions may be stated as follows. There is no satisfactory evidence that it is the general practice at the London Hospital to exclude cases of acute rheumatism in favour of any other disease. There is, however, some reason to think that in sub-acute cases a certain negative bias exists, and as no hard and fast line can be drawn between acute and sub-acute cases, this lessens to some extent the importance of the material from a

statistical point of view. We shall have occasion to point out later that the existence of such a bias *may*, perhaps, explain a curious discontinuity in our results, at present we merely note its possible existence as a source of error.

The very special character of a "General Hospital Population" cannot be too strongly emphasized. Apparent as is the fact, we find it constantly disregarded. Every year numerous papers are published in which various theories of pathogeny or methods of treatment are supported by appeals to hospital statistics. In general, the statistical methods employed in these writings are so inadequate that just conclusions could hardly be drawn; in the rare instances, in which this is not the case, the fundamental distinctions above drawn have been ignored, a regrettable waste of time and energy resulting.

We have already noted that acute rheumatism exhibits marked seasonal variations in frequency. It might, however, be objected that the discrepancies result from "errors due to random sampling," *i.e.* that if the numbers were larger, the monthly returns would be sensibly equal, on making allowance for the differences in length of the calendar months. That this is not the case seems to be proved by the following considerations.

If there be no bias in favour of any particular season, the number of cases occurring in any thirty-day month will be  $30/365$  of the total admissions. In other words, the chance in favour of any given case falling in such a month is  $30/365$ , and against the event,  $335/365$ . Consequently, if we know the total admissions for a year, or series of years, we can find the number which should, theoretically, fall in any month. Further, if the actual number admitted exceeds or falls short of this value, we can calculate the chance against such a deviation being merely a result of "random sampling." Thus, in a series of nine years, the number of females with acute rheumatism admitted to the London Hospital in November was 159. The theoretical number on the above hypothesis is 116.71 and the deviation 42.29. Obtaining the value of  $\sigma$  for the normal curve from the expression  $\sigma = \sqrt{npq}$ , where  $n = 1449$ ,  $p = 30/365$  and  $q = 335/365$ , we find  $\sigma = 10.93$  (approx.). Hence  $\frac{x}{\sigma} = \frac{42.29}{10.93} = 3.87$ , and the chance against such a deviation, *or a greater*, occurring is obtained by consulting a table of the probability integral (*see* Sheppard, 1903). This gives 9999456:544 or 18,381 to 1 against<sup>1</sup>.

<sup>1</sup> This case has merely been given as an example, we attach no importance to the actual figures.



## 174 *Meteorological Factors and Acute Rheumatism*

It is therefore clear that some seasonal bias does really exist. These facts are illustrated by the accompanying Diagram 1, which was constructed from fuller *data*. The total number of admissions for acute rheumatism to the London Hospital, from 1873—1903 (inclusive) was reduced to daily averages for each month, so as to render the admissions for different months comparable; a suitable correction was also introduced for Leap Year returns. The figures are as follows:—

January	...	...	·8054
February	...	...	·7460
March	...	...	·6639
April	...	...	·6785
May	...	...	·6347
June	...	...	·7688
July	...	...	·8262
August	...	...	·9095
September	...	...	1·1054
October	...	...	1·1197
November	...	...	1·1591
December	...	...	·9011

These figures are plotted on the diagram. It will be noted that they are in substantial agreement with those of Gabbett, drawn from a shorter series of cases admitted to the same hospital.

We next attempted to refer this discrepancy in monthly returns to meteorological variations, employing the process about to be described.

Since the total admissions to the hospital have increased from 1913 (medical admissions) in 1873 to 5196 in 1904, the actual numbers in the different years are not comparable. Hence, we must use as our measure of rheumatism frequency not the absolute numbers, but the ratios of these to the total (medical) admissions for the month, or, if the hospital be usually full, for the year. These ratios were accordingly calculated for the whole series of 372 months from 1873 to 1903.

As criteria of weather conditions we have used:—

- (1) The mean monthly rainfall (in inches),
- (2) The mean barometric height (in inches),
- (3) The mean temperature of the air (Fahrenheit),
- (4) Monthly mean degrees of humidity (saturation 100).

(1) was obtained from the paper by Nash (1904). For the *data* under (2), (3), (4) we are indebted to the courtesy of the authorities at the Royal Observatory Greenwich, who most kindly extracted the required information from their Annual Reports.

We had, therefore, two sets of variables, the monthly rheumatism ratios and (1), (2), (3) and (4), so that four series of correlation coefficients could be calculated<sup>1</sup>.



Total Monthly Admissions of Acute Rheumatism at the London Hospital from 1873 to 1903 (inclusive). [Reduced to daily averages for each month, so as to render the admissions for different months comparable: a suitable correction also introduced for Leap Years.]

DIAGRAM 1.

<sup>1</sup> It is not possible to discuss here the theory of correlation or methods of calculation; an elementary account will be found in Karl Pearson (1900), *Grammar of Science*, 2nd ed. London, pp. 392 et seq. The coefficient of correlation ( $r$ ) is a measure of relationship between two variables, it may take any value between 0 and 1. When it is zero the variables are unrelated; when it is unity the relationship is perfect (under "normal" conditions). If the coefficient be positive, the variables increase together; if negative, as one increases, the other decreases.

The "probable error" must always be ascertained. *A coefficient less in magnitude than twice its "probable error" is certainly, and one less than three times its "error" probably, quite without significance.*

## 176 *Meteorological Factors and Acute Rheumatism*

This has been done for the years 1873—1903, with the tabled results. As we had only 31 observations, the moments and products were obtained by referring each individual value to the axes, without forming a correlation table, thus avoiding errors of grouping.

TABLE I.

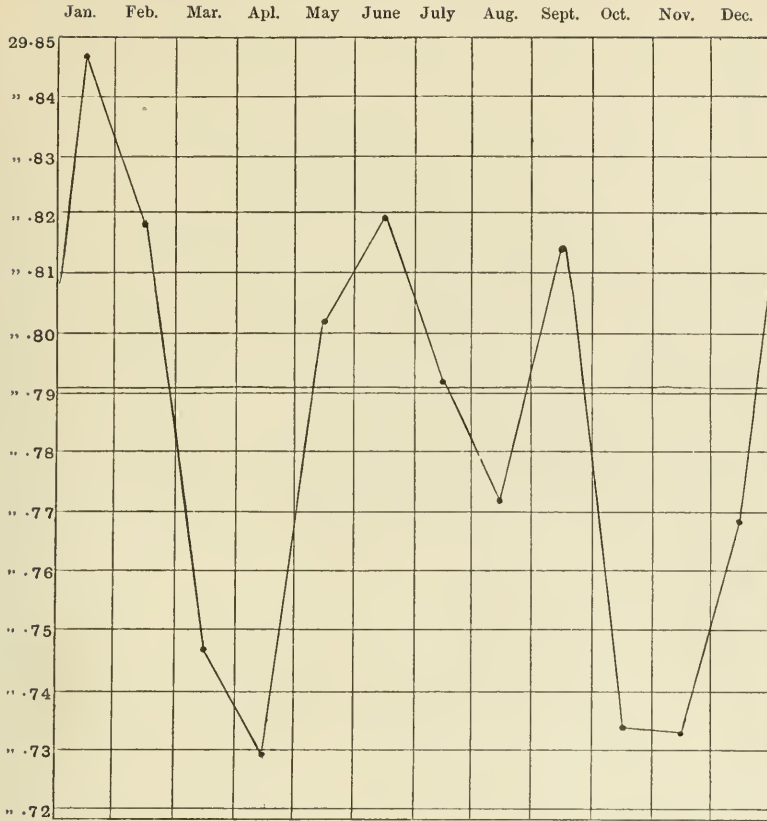
### *Rheumatism Ratios and Rainfall.*

Month	Rainfall (in inches)		Rheumatism ratios		Correlation <i>r</i>
	Mean	Standard deviation	Mean	Standard deviation	
January	1·66	·96	·00665	·00236	·2447 ±·1139
February	1·56	·93	·00550	·00171	·1260 ±·1192
March	1·46	·76	·00533	·00119	−·1210 ±·1194
April	1·59	·88	·00535	·00146	·03844 ±·1210
May	1·75	1·05	·00509	·00139	−·1385 ±·1188
June	2·11	1·23	·00605	·00160	·0507 ±·1208
July	2·45	1·51	·00668	·00192	·1441 ±·1186
August	2·38	1·30	·00719	·00191	−·4720 ±·0942
September	2·03	1·13	·00844	·00225	·2375 ±·1143
October	2·72	1·56	·00912	·00278	·2595 ±·1131
November	2·28	·93	·00928	·00244	·01509 ±·1211
December	1·96	1·06	·00748	·00196	−·00153 ±·1211

TABLE II.

### *Rheumatism Ratios and Mean Barometric Height.*

Month	Barometer (in inches)		Correlation with Rheumatism Ratios
	Mean	Standard deviation	
January	29·847	·1914	·0874 ±·1212
February	29·818	·2131	−·1113 ±·1196
March	29·747	·1546	·0816 ±·1203
April	29·729	·1074	·03282 ±·1210
May	29·802	·1048	·2614 ±·1129
June	29·819	·0708	·1827 ±·1171
July	29·792	·0852	·2807 ±·1117
August	29·772	·0759	·3576 ±·1056
September	29·814	1·040	−·2381 ±·1143
October	29·734	·1370	−·1374 ±·1189
November	29·733	·2709	−·09413 ±·1201
December	29·768	·1902	·1446 ±·1186



Mean Barometric Height in inches for the years 1873 to 1903 (inclusive).

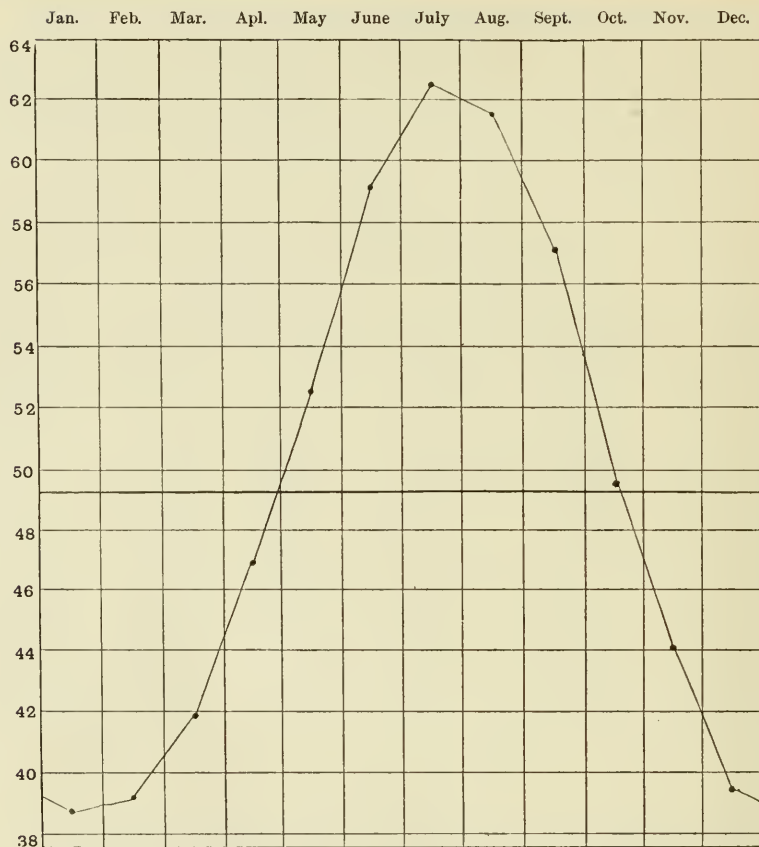
DIAGRAM 2.

TABLE III.

*Rheumatism Ratios and Mean Temperature.*

Month	Temperature (in degrees, Fahrenheit)		Correlation with Rheumatism Ratios
	Mean	Standard deviation	
January	38.736	3.64	.2457 $\pm$ .1138
February	39.339	3.55	.1233 $\pm$ .1193
March	41.86	2.73	-.05092 $\pm$ .1208
April	46.87	1.89	.1375 $\pm$ .1188
May	52.59	2.59	.0527 $\pm$ .1208
June	59.17	1.71	.0789 $\pm$ .1204
July	62.46	2.49	.1757 $\pm$ .1174
August	61.58	2.01	.1378 $\pm$ .1188
September	57.18	2.13	-.0790 $\pm$ .1204
October	49.47	2.44	-.1320 $\pm$ .1193
November	44.05	2.44	-.2324 $\pm$ .1446
December	39.43	3.54	.0821 $\pm$ .1203

# 178 *Meteorological Factors and Acute Rheumatism*



Mean Monthly Temperature in degrees Fahrenheit from 1873 to 1903 (inclusive).

DIAGRAM 3.

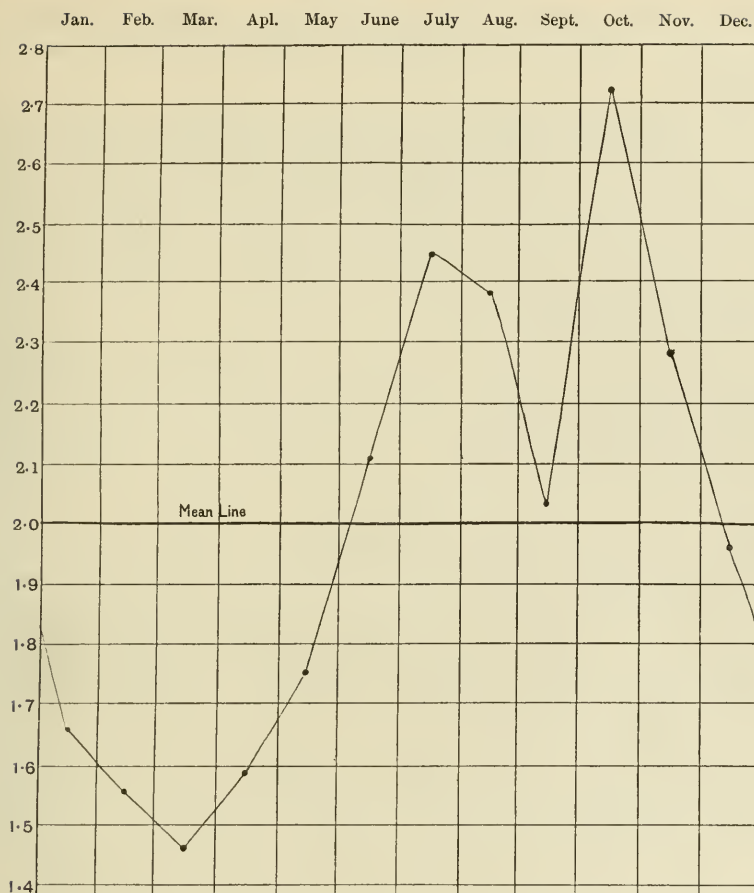
TABLE IV.

## *Rheumatism Ratios and Mean Humidity.*

(Only a few of these are given, as the percentage saturation values are of questionable utility from the present standpoint.)

Month	Percentage saturation		Correlation with Rheumatism Ratios
	Mean	Standard deviation	
March	79.85	2.59	-.2636 ± .1127
May	74.11	3.37	-.1845 ± .1170
June	73.98	3.90	-.0612 ± .1207
August	75.93	4.21	-.1935 ± .1166
September	80.37	3.71	.1701 ± .1176





The Mean Monthly Rainfall in inches for the years 1873 to 1903 (inclusive).

DIAGRAM 4.

A comparison of the correlation coefficients with their probable errors shows that, with two exceptions, the results are negligible. Except for the month of August no relation between meteorological conditions and rheumatism can be inferred from our investigation.

It might be suggested that owing to the interval separating the development of the disease and admission to hospital, a relation should rather be sought between the rheumatism returns for one month and the weather variables for the preceding month. We have tested this for the month of August.

The correlation between August rheumatism ratios and July

rainfall is  $\cdot0087 \pm \cdot1211$ : between the former and July humidity,  $\cdot0606 \pm \cdot1207$ : with July temperature  $\cdot0182 \pm \cdot1211$ . These are all negligible.

As we have said, there is one peculiar exception to the general results, namely the rainfall and barometer correlations for August. In this month alone, we obtain a significant negative correlation between rheumatism ratios and rainfall, a substantial positive correlation between the former and barometric height. The inference being that rheumatism is associated with dry weather as upheld by Newsholme and, to some extent, Příbram.

It is not easy to see why the month of August should occupy a unique position in our return. Meteorologically, this month is not sharply contrasted with its successors or fore-runners in any respect, nor are the rheumatism admissions maximal. It is possible that the explanation is afforded by the selective character of hospital admissions. The factors which have been *asserted* to militate against the admission of rheumatism to a general hospital (necessarily long occupancy of a bed, etc.) would be least operative in a holiday month, when little clinical teaching is given and few "requests" are sent up owing to the usual absence of the senior staff. If this be the case, this month alone yields unbiassed statistics and the positive results obtained are of special interest as probably containing a general truth as to the aetiology of acute rheumatism; but as the explanation depends upon assumptions, the accuracy of which we have not been able to establish, it must be regarded as a pure speculation. To avoid any possible misconception we repeat here that no trustworthy evidence is known to us from which we can infer that a *definite* selection of rheumatism exists at the London Hospital.

Apart from the errors dependent on the peculiar material we have analysed, it may be doubted whether the methods employed are sufficiently delicate. We can only say that, although not ideal, they appear more exact than any other readily available. Thus, a more satisfactory method in theory could be founded on a consideration of the curve of rheumatisms given in the first diagram. This appears to resemble closely a compound harmonic curve. It might therefore be analysed by a mechanical harmonic analyser and its components compared with the periodic rainfall curve. We hoped to be able to make some statement as to this matter, but the necessary instrument and power to use it not being at our disposal, we have employed the method above described. Indeed, we are not satisfied that more definite results would have been obtained.

## CONCLUSIONS.

Summing up, our conclusions may be expressed as follows:—

1. Acute rheumatism admissions in a "General Hospital Population" exhibits significant seasonal variations.
2. There is evidence of a connection between rheumatism incidence and dry weather for one series of months.
3. The failure to obtain definite results in a majority of the returns is probably dependent on the special nature of the material and suggests that a satisfactory solution of the problem cannot be obtained from hospital statistics.
4. Statistical results obtained from material of this type cannot be applied without further consideration to a normal population.

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SOME EXPERIMENTS WITH FLUORESCEIN AS AN  
AGENT FOR THE DETECTION OF POLLUTION  
OF WELLS.

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(1 Map.)

MOST towns augment their water supply from wells, and in South Africa, where the rivers are said to run underground, Johannesburg is no exception.

Its chief supply is from deep wells in the dolomite some 20 miles to the south-west of the town, but one of the suburbs—Parktown—is not connected to the main supply, but at present draws its water from two wells (the property of the Rand Water Board) which will be subsequently referred to as No. 1 and No. 2, sunk in the shales of the upper Witwatersrand beds. From here the water is pumped to two Water Towers situated at the highest point of the suburb from whence it is distributed by gravitation. For various reasons, the Rand Water Board had decided on replacing the pumping machinery at these wells by electrically driven gear, and advantage was to be taken of the change to properly line and deepen the wells by a steel bore-hole.

Chemical and bacteriological samples regularly taken had shown the water to be of great purity chemically, but *B. coli* had occasionally been found, their presence, however, being attributed to temporary and accidental contaminations due to the entrance of workmen to the wells, etc.

Owing to an outbreak of Plague and other circumstances, no regular sampling was done between March 1904 and May 1905. On resumption, however, the results, both chemically and bacteriologically, were very satisfactory. This condition continued until December, 1905,

when a sample taken from one of the Water Towers showed a marked increase in the albuminoid ammonia figure, and was described as "dirty."

From this time on to March 1905, samples taken from various taps, whilst chemically above reproach, contained *B. coli* in 10 c.c. or less.

Suspicion attached to the Water Towers, which were thoroughly cleansed and disinfected, but on March 19th the number of *B. coli* and other organisms growing in cultures at body-temperature, had increased in No. 1 well, and it was concluded that any pollution must be getting access to the well water itself.

(It is interesting here to note that between April 1905 and May 1906 only two cases of water-borne disease, viz., one of enteric and one of dysentery, occurred in the suburb.)

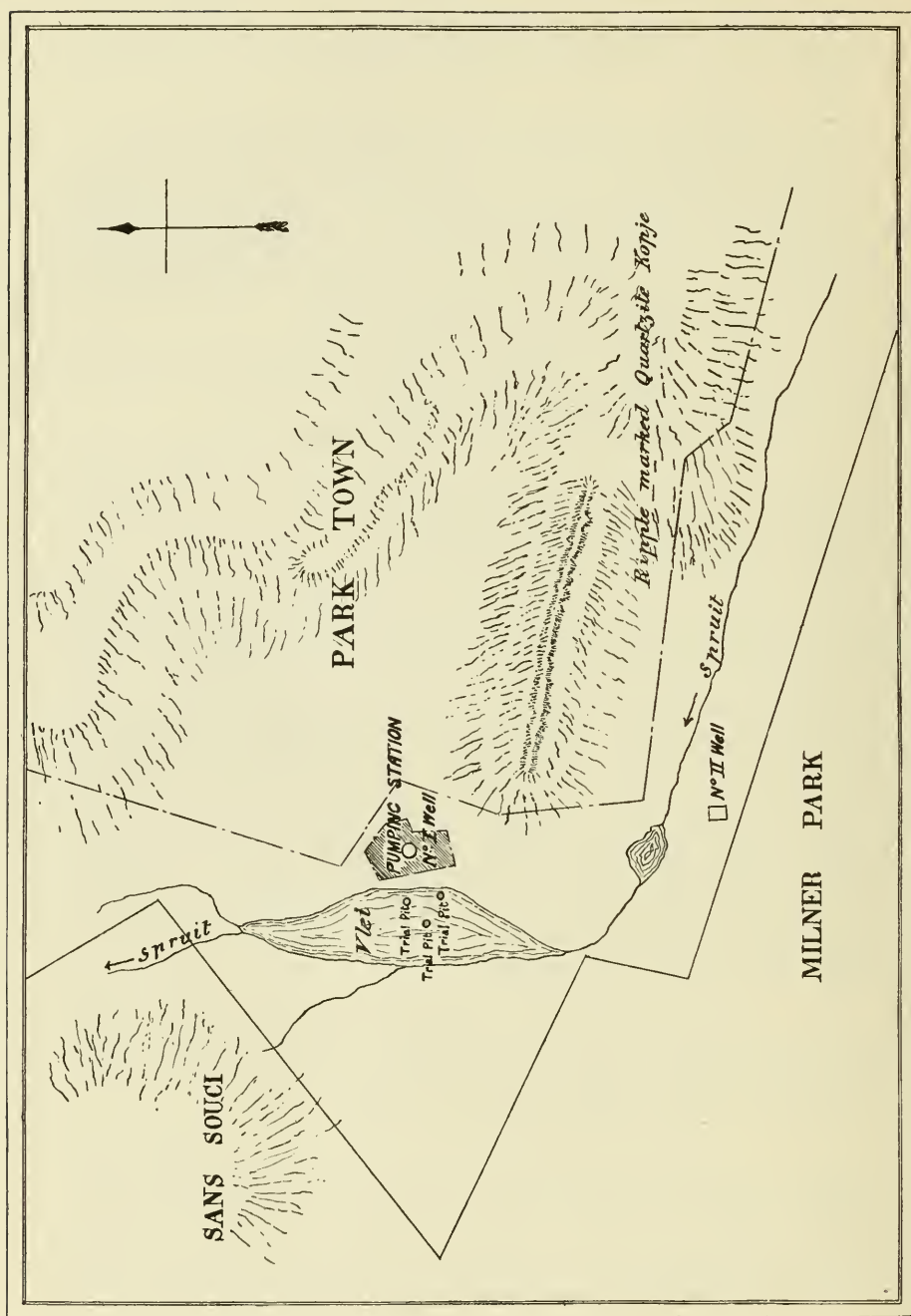
About this time it came to our knowledge that after long continued pumping from the wells, the level of the water in the adjoining spruit was lowered, but in order that this fact may be better understood, a brief description of the wells is necessary.

No. 1 and No. 2 wells are sunk in weathered red shales—which dip from north to south—through a tract of alluvium, the latter being traversed by the bed of a spruit (or water-course), the water of which is often much polluted, and which courses from Parktown Gates westwards between the ripple-marked quartzite kopjes and the sloping ground on Milner Park. Passing some 50 yards to the north of No. 2 well, the stream turns north and flows about 100 yards to the west of the Pumping Station. Augmented between No. 2 well and the Station by some minor springs in wet weather, it overflows on to a strip of marshy ground, or "Vlei," which lies between it and the Pumping Station. On the eastern, or pump-house side of the Vlei, is an intermittent spring known as the "Peach Tree Eye" from its proximity to a tree of that name.

No. 1 well is situated within the pump-house, and is 75 feet deep, with two tunnels at the lower end respectively 23 and 30 feet long. The yield is about 100,000 gallons per day. There is no proper lining, planks and timber baulks having been used.

No. 2 well is some 200 yards to the south of the Engine House and is also sunk through alluvium in the weathered red shales; the supply is apparently superficial and flows into the bottom of a rectangular basin some 16 feet square by 15 feet deep, with cemented sides and enclosed by a non-dustproof corrugated iron structure. The water enters chiefly near the south-eastern corner, and except to occa-





sionally augment the supply, is little used for drinking purposes, being used for boilers, etc.

Careful inquiry and inspection had corroborated the statement that the level of the water in the adjoining spruit was lowered after heavy pumping, and it seemed probable that pollution was gaining access to No. 1 well from the Spruit and Vlei, and that it was due to reversed flow or insuction, when the level of the well water was lowered by pumping, along the channel of natural overflow.

It was determined to test this hypothesis, and from some previous work undertaken, fluorescein was selected as the agent. After some preliminary trials, the following experiments were started on April 4th.

The amount of fluorescein at our disposal being small (about 50 grams) the area of operation was restricted to three trial pits, each about 6 or 7 feet deep and 3 feet in diameter, sunk in the marshy ground near the Peach Tree, where the spring had been noticed. To the water *which was present* in the pits a solution of fluorescein was added.

The height of the water in the well and trial pits having been carefully noted, pumping from the former, using extra gear, was started at 12.45 p.m., arrangements having previously been made to collect samples from the well at frequent intervals.

The water in the well having fallen some 45 feet, pumping was stopped at 7 p.m., when it was found that the water level in the three trial pits had fallen  $14\frac{5}{8}$ ,  $11\frac{7}{8}$ , and  $4\frac{1}{2}$  inches respectively.

No doubt this of itself showed a possible connection, but on ordinary examination, fluorescein could not be detected in any samples. About two litres of each sample were therefore concentrated by boiling in a glass dish to about 10 cubic centimetres, and after filtration from the solid deposit, fluorescein was detected in the filtrate of the sample collected on the third day. Possibly the concentration would have been unnecessary had we been able to use more fluorescein in the first place.

The connection between the Vlei and Well being thus established, pumping was discontinued, and the Town Mains connected to the Water Towers.

Arrangements were then made for thoroughly overhauling the well, and on the 18th April, after the pumps had run off the water for some time, a descent was made, when a fall of rock in the western face, some 30 feet down, was discovered, leaving a chasm passing upwards and to the west some 14 feet long. From its appearance, the fall was

of fairly recent date, and no doubt by opening up fissures, etc., had permitted water from the marsh to gain access practically unfiltered to the well.

An opportunity was afterwards afforded of taking samples from the water as it entered the drives, and were found of excellent quality, and since the work undertaken by the Rand Water Board in connection with the well has been completed, all trace of pollution has ceased, and the weekly samples, both chemically and bacteriologically, are quite satisfactory.

Experiments were afterwards carried out with No. 2 well, the surrounding ground being alluvium over the disintegrating shales and a good natural filter.

On April the 20th, fluorescein was put down in a disused well shaft some 80 yards to the east of the well, in what appeared to be a swallow hole about 50 yards north-east, and in a trench 6 feet from the well coping. In the first two the solution was added to the water already standing; in the last, poured into the trench. Pumping was started, but the samples were taken from the basin itself.

On April 26th, the same procedure having been followed with regard to the examination of the samples, it was thought that fluorescein was present in the basin, but as the amount must have been very small, more fluorescein was put down on April 30th. Samples taken the next day showed distinctly the presence of fluorescein. It was still present on May 8th, though in lessened amount, but following a heavy shower the previous day, again appeared on May 21st.

As has been stated before, the sides of the basin were of cement and practically water-proof, the water chiefly entering at the south-east corner. The shortest course for the fluorescein to have travelled would therefore have been about 18 feet, and the time six days.

At this time, apart from the article in the *Standard Dictionary*, the literature at our disposal contained hardly a reference to fluorescein, but the experience gained from the experiments themselves, suggested the need for further investigations, the preliminaries for which were being arranged when, on April 26th, 1906, Dr Porter directed our attention to a copy of Dr Copeman's report "On the outbreak of Enteric Fever at the Fulbourn Asylum," which he had just received through the courtesy of Mr W. H. Power, C.B., F.R.S., of the Local Government Board. While our own conclusions were almost in accord with Dr Copeman's, we freely availed ourselves of the information contained in his report in the following experiments:—

From some preliminary work it had been found that on passing a 1 in 550,000 solution of fluorescein in water through a column of soil, the fluorescein was at first completely removed, but as filtration proceeded, the filtrate was found to contain fluorescein, and ultimately the solution passed through undiminished in fluorescing power. Prolonged percolation of water showed that the fluorescein could be completely washed out of the soil.

By substituting a fine grained sand for the soil, exactly the same phenomenon was observed and further, it was found that precisely the same results were obtained when use was made of a solution of fluorescein made alkaline with sodium hydroxide.

In order to ascertain if the extent of the removal of the fluorescein from solution was proportional to the amount of sand through which the solution filtered, three tubes of the same internal diameter (1.9 cm.) were fitted with stoppers through which passed narrow glass tubes. A layer of coarse gravel—about 3 cm. deep—was placed in each tube, and on this were placed columns of fine sand respectively 7 cm., 14 cm., and 27 cm. in length. These tubes were set vertically, and flasks containing a solution of one part of fluorescein in 200,000 of water were arranged over them in such a way that the solution was delivered into the tubes about 7 cm. above the surface of the sand; the level and hydrostatic pressure therefore being constant throughout the filtration and was approximately the same in each tube.

The following table shows the results:—

Length of sand column	...	...	...	7 cm.	14 cm.	27 cm.
Time taken from starting filtration until 1st drop fell from the narrow tube	...	...	...	17 min.	35 min.	90 min.
Volume of filtrate before fluorescein made its evident appearance	...	...	...	9 c.c.	17 c.c.	36 c.c.
Time taken for the solution to pass through unchanged (estimated approximately)	...	...	...	115 min.	180 min.	350 min.

These results indicate that the amount of fluorescein removed was proportional to the amount of sand with which the solution came into contact, and taken in conjunction with the fact already established, namely, that the removed fluorescein could be washed out of the sand, suggests that the fluorescein is mechanically adsorbed by the sand or soil, and probably the adsorption proceeds until an equilibrium is established between the concentration of the fluorescein in the film adhering to the surface of the grains and that in the bulk solution. This view—that the phenomenon is a surface one—is supported by



the fact that when coarse sand (presenting a smaller surface) was used a smaller amount of fluorescein was adsorbed. The process would appear to be comparable with the dyeing of wool with substantive dyestuffs.

Copeman states that by shaking up ground chalk with a solution of fluorescein, the intensity of colouration was not diminished. As we were unable to procure chalk we could not repeat this, but on shaking up a distinctly fluorescing solution with sand we were unable to satisfy ourselves that any diminution of fluorescein resulted; nevertheless, it is highly probable that if a solution of fluorescein was passed through a column of chalk adsorption would take place.

For the recognition of fluorescence caused by fluorescein we prefer to examine the solution against a dark background, rather than against the white one suggested by Copeman: a good light is necessary, and the solution should not be placed too near the dark surface. Examination of the solution by magnesium light, while a good means of recognising the fluorescence, does not appear to possess any special virtue, or surpass good daylight.

Copeman states that fluorescence is appreciable in a dilution of one in 100,000,000. Obviously the delicacy of the recognition can be increased by concentrating the solution suspected to contain fluorescein provided that no change takes place on evaporation. In order to test this point, a solution of fluorescein, containing one part in 50,000,000, was prepared; in this solution the fluorescence was just recognisable. 10 c.c. of the solution were made up to 2000 c.c. with (1) distilled water, and (2) Johannesburg tap water. At this high dilution (one part in 10,000,000,000) the fluorescence could not be recognised. The dilute solutions were concentrated on the water-bath to 10 c.c., and when examined in an ordinary Nessler tube (giving a depth of liquid of about 2 cm.), the solution showed fluorescence comparable in intensity with the original solution (1 in 50,000,000). When concentrating in this way, a small basin should be used, and filled up with the solution under examination as the bulk is reduced by evaporation: by this means the fluorescein is kept in solution, and does not deposit on the side of the vessel as it would if a large basin was used. Evaporation to dryness on the water-bath although not advisable, does not seem, however, to be prejudicial, for on moistening with water the fluorescein readily passes into solution.

In these laboratory experiments it was not found necessary to render the solution of fluorescein alkaline, probably because sufficient



alkali had been dissolved from the glass—in actual practice, however, it is advisable to do so, since in acid solution fluorescein does not give a characteristic green fluorescence.

One of us has recently had an opportunity of using the concentration method for tracing the course of underground streams over considerable distances, the method proving of great service. In this instance the water at the point of reappearance assumed no obvious colour, but by concentrating, the presence of fluorescein was definitely established.

In connection with the use of fluorescein for detecting a water contamination, it has been suggested that the method is of little use, because it does not follow that bacteria can go where fluorescein can. But this is an objection which, if valid, applies with equal force to all methods wherein the contaminating connection is sought by means of any substance in solution (cf. P. F. Frankland's method with a lithium salt). We are, however, not prepared to admit the validity of this objection; if a solution of fluorescein put into a hole or well in the ground finds its way to another hole or well some distance off, this is definite evidence that a water-connection exists between the two. There is a possibility that the soil between acts as an efficient filter, but this possibility cannot be relied upon, for the connection may be either (1) an underground "lake" of water (as at the Fulbourn District, Cambridge), or (2) a flaw in the rock formation which is so loosely filled with soil, or débris, that it cannot act as an efficient filter.

In so vital a matter as a water-supply therefore, we hold that if a connection can be established by means of fluorescein between a spot known to be contaminated and the source of the water supply, such supply should be regarded as dangerous, and liable at any time to give rise to a water-borne epidemic.

In view of the adsorbing action which soils and sands have been shown to exert on fluorescein, it seemed desirable to ascertain if bacteria would percolate through a column of soil in a similar manner to fluorescein.

A preliminary experiment with an organism of the *B. prodigiosus* group led to no satisfactory result, but more success was obtained with a good pigment-forming strain of a green, very motile organism, closely akin to the *B. pyocyaneus*. A number of trials showed that this organism could be easily recovered both from distilled and tap water, even after some days, and as the presence of fluorescein did

not interfere with its recovery, the organism was suitable for our purpose, and the following experiments were carried out:—

A tube, similar to that previously described, was prepared with a column of fine sand 7·5 cm. in length, and the sand, apparatus, etc., having been found free from the pigment-producing organism, 50 c.c. of a 0·02% solution of fluorescein was made up to 1000 c.c. with distilled water, to which a small quantity of an agar growth of the organism had been previously added and incubated for 24 hours: the solution being fed on to the sand as in previous experiments.

Sixteen minutes elapsed before the first drop fell from the tube, the observations made being as follows:—

	Fluorescence	Green organism
First 0·5 c.c.	None	Present
Next 5·5 c.c. (15 mins. for the collection of 6 c.c.)	Very faint trace	„
7th c.c.	Faint trace	„
8th—17th c.c.	Trace	„
18th c.c. (64 mins. for collection of 18 c.c.)	Very distinct	„

In the 19th c.c. the fluorescein solution came through apparently unchanged.

	Fluorescence	Green organism
19th—28th c.c.	Unchanged	Present
119th c.c.	„	„

Altogether about 500 c.c. of the solution were passed through the sand column. After the fluorescein solution had drained, water was passed through the column, and the green organism was found to be present in the 1st, 50th, 100th, 260th, and 660th cubic centimetre of the wash-water. It was again found that the fluorescein was washed out of the sand.

An experiment carried out similarly with the same material, but with a column 32 cm. long, gave the following results: 69 minutes elapsed until the first drop fell from the narrow tube.

	Fluorescence	Green organism
First $\frac{1}{2}$ c.c.	None	Present
11th c.c.	„	„
21st c.c.	Faint trace	„
22nd to 30th c.c.	Present	„
31st c.c.	Distinct	„
41st c.c.	Very distinct	„
51st c.c.	Apparently unchanged	„
832nd c.c.	„	„

After complete draining, water was added, and the green organism was found in the 7th c.c. of the washing, showing that although the experiment had lasted some days the sand column had not become a *filter* for the organism.

With a column of sea-sand 32 cm. long the first drop came through in  $5\frac{3}{4}$  minutes, and showed a very slight (but very greatly diminished as compared with the original solution) fluorescence. After about 4 c.c. had filtered, the fluorescence was apparently as great as in the original solution. The green organism was proved to be present in the 1st, 61st, 110th, and 500th c.c. When a 32 cm. long column of a very sandy soil was used, the first drop fell after six minutes five seconds, and hardly showed any fluorescence. The fluorescence of the first c.c. was not so strong as in the first c.c. which passed through the column of sea-sand. After about 45 c.c. had passed through the intensity of the fluorescence was about the same as in the original solution. The green organism was found in the 1st, 11th and 100th c.c.

A black loamy soil (from Potchefstroom) was next tried. Through a column 32 cm. long the percolation was very slow: 6 hours and 43 minutes elapsing before the first drop fell. Contrary to expectation, however, the first drop showed a very faint fluorescence. The faint colouration persisted for a considerable time without apparently becoming more distinct: after about 20 c.c. had passed through, the intensity of fluorescence began to increase, and after about 50 c.c. had percolated, the intensity was about as great as in the original solution. There appeared in this case to be a slight filtering action with respect to the organism, for on plating the first c.c. the pigment did not show until considerably after 24 hours' incubation. This may, however, have been due to the presence of some substance extracted from the soil (humic acid?) inhibiting the growth or pigmenting power of the organism. The organism was similarly found in the 11th c.c. and in the 30th c.c., showing on agar after 24 hours' incubation.

### *Conclusions.*

These results, and particularly those noted in connection with adsorption, indicate that fluorescein must not be expected to appear in a time proportional to the rate of flow when the water has to percolate through soil, gravel, sand, or detritus. Its appearance will be the longer delayed the finer the material through which the water passes.

Evaporation of the water renders the detection of fluorescein more delicate, but no attempt has been made to show to what extent this is practicable.

The experiments show that  $2\frac{1}{2}$  litres can be concentrated for this purpose, and this has proved to be a convenient quantity.

Concentration should not be carried too far, the best results being obtained when the volume was not reduced below 5 c.c. It will usually be found that the concentrate has to be filtered, and in this connection it must be remembered that filter-paper exerts an adsorbing action, consequently, when the filter has drained, it is advisable to wash the paper with 1 or 2 c.c. of water. When the water contains iron, care must be taken not to confuse, in the concentrate, the greenish colour due to the presence of this metal with the green tinge of fluorescein. Until some experience is gained, it is advisable to use, as a delicate control, a very dilute solution of fluorescein.

The Concentration Method has the further advantage of lessening the amount of fluorescein needful, as compared with that necessary to impart an obvious fluorescence to the water as it appears at its point of recovery. This may be of importance when, for aesthetic reasons, it is not advisable to add so much fluorescein that a stream will become visibly coloured—although in the quantity necessary to produce this the material is harmless.

In conclusion, we desire to express our thanks to Dr Porter (Medical Officer of Health, Johannesburg), under whose direction the actual experiments at the wells were carried out, and of whose report on the subject we have freely availed ourselves, for permission to publish the first portion of this paper, and to Mr Louttit (Bacteriological Assistant at the Government Laboratories) for assistance in the bacteriological portion of the work.

# ON VARIATIONS OF THE MENINGOCOCCUS AND ITS DIFFERENTIATION FROM OTHER COCCI OCCURRING IN THE CEREBRO-SPINAL FLUID.

BY J. A. ARKWRIGHT, M.D.

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WEICHSELBAUM (1887) first described his *Diplococcus intracellularis meningitidis* (meningococcus) as having been cultivated within a few hours after death from the brain or meninges of six cases of acute cerebro-spinal meningitis. These cases occurred during the years 1885—1887 at Vienna, when no regular epidemic existed. Cases had however occurred from time to time, and there was an epidemic in part of Lower Austria in 1886. The youngest case was 14 years old. He described the micrococcus as being of a wide gonococcus-like shape, occurring in pairs or fours, not in chains, negative to Gram's stain. It failed to grow at 20° C., but grew well on agar at 37° C. almost exclusively on the surface. The growth was viscid, the colonies were often confluent and appeared finely granular when slightly magnified. The organisms required sub-culture every few days to ensure success, were easily killed by drying, and showed very little growth in broth.

Jaeger (1895) described a diplococcus associated with an epidemic of cerebro-spinal meningitis. He stated that it was similar to Weichselbaum's organism but that after a short period of cultivation in artificial media it changed its character, becoming Gram-positive, and occasionally grew in short chains and became resistant to drying.

Councilman, Mallory and Wright (1898) found Weichselbaum's diplococcus in an epidemic in Massachusetts, and confirmed the persistence of its Gram-negative characteristics and its susceptibility to drying.

Still (1898) cultivated the meningococcus from a series of cases of posterior basic meningitis, and found that it agreed very closely with Weichselbaum's description, but that it was a little longer-lived in cultures, and grew rather more readily on artificial media.



Albrecht and Ghon (1903) appear to have proved that Jaeger's meningococcus is another organism which appeared in his cultures, although he probably found the true meningococcus of Weichselbaum in his original preparations.

Various statements have been made about the resistance of the meningococcus to drying. Germano (1897) stated that meningococci mixed with dust and dried lived 80—90 days, but he was working with Jaeger's strain, which is not now regarded as the true meningococcus. Councilman, Mallory and Wright (1898) dried meningococci on sterile paper in Petri dishes and placed these in a dark drawer at room temperature. They succeeded in obtaining cultures from the dried material after 60 hours but failed to do so after 72 hours. Albrecht and Ghon (1903) dried a meningococcus emulsion on coverslips and kept them for 24 hours at room temperature in the dark. After this the cocci were dead. Bettencourt and França (1902) found meningococci alive after six hours, but not after nine hours' drying on cotton threads at room temperature in the dark.

v. Lingelsheim (1906), during the great epidemic in Upper Silesia, found that material obtained by lumbar puncture or post-mortem or from the nose, yielded meningococci more frequently if the specimen was received and cultures were made at the laboratory within a few hours of its removal from the body or the death of the patient.

In the case of 31 post-mortem specimens where special precautions were taken to promote rapidity of transit, the meningococcus was isolated from every one. This observer carried out experiments showing that the meningococcus is not affected by diffuse light though killed by direct sunlight. He is also of the opinion that cultures live a shorter time at low temperatures only because they do not grow and the individual life of the coccus is always short. He found that the meningococcus would survive a temperature of  $-10^{\circ}\text{C}$ . or  $-20^{\circ}\text{C}$ . for two hours.

It has been stated that the meningococcus is especially susceptible to cold, but the above experiments disprove this.

v. Lingelsheim expresses the opinion that the Gram-negative cocci found by him inside leucocytes in cerebro-spinal fluid in cases of meningitis were always true meningococci. He tested the reactions of cultures of various Gram-negative cocci grown on sugar-containing media, and found that the meningococcus produced acid on dextrose and maltose, but not on laevulose, galactose, mannite, dulcite, cane sugar, lactose or inulin. Indeed he makes the production of acid from laevulose a proof that the organism is not a true meningococcus, but as he used

solid media and took the reaction after 24 hours as his criterion his results are hardly comparable with that of Gordon. The different reactions of cultures and Gram-negative cocci in broth containing various carbohydrates as given by Dunn and Gordon (1905) are detailed in a paper by me (on the occurrence of *Micrococcus catarrhalis* and its differentiation from other Gram-negative cocci) in this *Journal* (Jan. 1907).

C. Fraenkel (1905) found that meningococci made a feeble growth on gelatin at 22° C., but not at 20° C.

Other cocci found in the cerebro-spinal fluid drawn off by lumbar puncture, are of special interest on account of the difficulty there may be in differentiating them from the meningococcus, and also because some of them occur associated with the meningococcus when this organism may nevertheless be considered the true or chief cause of the disease. The pneumococcus has long been known to be a cause of primary meningitis. Cases of this nature are described by Weichselbaum (1887). The pneumococcus is not infrequently associated with the meningococcus.

v. Lingelsheim (1906) enumerates the following organisms in addition to the *Pneumococcus*, as being found by him in the central nervous system in epidemic meningitis, viz.:—varieties of *Streptococcus*, *Staphylococcus*, *Diplococcus crassus*, *D. mucosus*, *D. pharyngis*, *flavus II*, and *Micrococcus cinereus*. The last three organisms are Gram-negative, but are distinguishable by the changes they produce in sugar-containing media, by their other cultural characters, and by agglutination tests.

*Diplococcus crassus* is of special interest, as v. Lingelsheim considers it identical with Jaeger's meningococcus. It is partly Gram-positive and partly Gram-negative; even cocci from adjacent colonies varying in the degree with which they retain the stain. It readily produces acid from several carbohydrates, including saccharose and lactose, and an antiserum obtained by injecting it into a rabbit does not agglutinate the meningococcus. v. Lingelsheim attributes certain symptoms in some cases of meningitis to an infection with *Diplococcus crassus*, added to that with the meningococcus.

Intracellular cocci in cerebro-spinal fluid may be the *Staphylococcus aureus*, as in a case recorded by Wright and Archibald (1906).

Agglutination experiments with the meningococcus have been recorded by many writers.

Bettencourt and França (1902) examined by the macroscopic method the agglutinating power of the blood serum of persons who were ill

with epidemic meningitis and of those who had recovered. As a control they tested the serum of 17 persons in health or suffering from other diseases. The serum from none of these latter agglutinated the meningococcus in dilutions of 1—10, and one doubtfully in those of 1—5.

In all the six patients suffering from meningitis whose serum they examined agglutination was obtained in dilutions varying from 1—10 to 1—100: in three cases as early as the 4th day, and in one as late as the 56th day. In a case examined on the 4th day the serum agglutinated at 1—50, whereas meningococci could not be found in the cerebro-spinal fluid till the 6th day. They tested the serum of 15 patients who had recovered and found that it still agglutinated in dilutions varying from 1—5 to 1—1000; 4 reaching 1—100 from 8 to 14 months after the onset of the disease.

v. Lingelsheim (1906) obtained agglutination of the meningococcus with the serum of patients in about 55% of cases examined on the 6—20th day, and in about 25% of those examined in the first 5 days or after the 20th day of the illness.

Both of these authors also obtained sera of high agglutinating values by injecting rabbits.

Goodwin and Sholly (1906) found that the agglutinating power of serum prepared by injecting meningococci varied much from day to day, and Dunham (1906) failed to obtain a serum of high agglutinating power.

Kutscher (1906) describes a strain of meningococcus agglutinated macroscopically by a specific horse serum, at 55° C., but not at 37° C.

The meningococcus may then be described as essentially a Gram-negative coccus, morphologically closely resembling the gonococcus and *M. catarrhalis*. It grows on agar at 37° C. after one or two cultures on serum-agar, as smooth or finely granular slightly milky translucent colonies, but does not grow on gelatin at 20° C. It forms acid in maltose and glucose broths, and as a rule in galactose and laevulose, but not in cane sugar. It is very easily killed by drying, and can be agglutinated by a specific serum prepared from a rabbit or horse.

I have cultivated the following strains:

Meningococcus XII, obtained from Dr Eyre.

Meningococcus XVII, kindly given me by Dr F. W. Andrewes. It was derived from a very rapidly fatal case of meningococcus septicaemia.

Meningococcus XIX, kindly given to me by Dr Graham Forbes, who isolated it from a very acute and rapidly fatal case of meningitis in a child.

I have also examined several specimens of cerebro-spinal fluid drawn off during life from patients suffering from meningitis. The cerebro-spinal fluid was poured on to sloped mixtures of agar and blood in tubes, and any deposit after centrifugalising was smeared on similar tubes. One post-mortem specimen of exudation on the meninges is included here.

*Specimen 1.* (From which strain XVIII was cultivated.) Cerebro-spinal fluid obtained by tapping the lateral ventricles of a child of 6 months old, who had been ill for 6 weeks. This child had a typical form of posterior basic meningitis and was an in-patient at St Bartholomew's Hospital under the care of Dr Morley Fletcher, who kindly gave me permission to examine the fluid. The diagnosis was confirmed by the post-mortem. The fluid was colourless not at all turbid and there was no deposit after centrifugalising. No cells or cocci were seen in the smear. Typical colonies appeared on blood-agar. The first cultures also contained streptococci.

*Specimen 2.* (From which strain XXIX was derived.) Cerebro-spinal fluid drawn off by lumbar puncture on the 5th day of illness from a child aged 6 months, under the care of Dr W. Carr at the Victoria Hospital, Chelsea, to whom I am indebted for the material. This was a case of posterior basic meningitis, confirmed post-mortem. The fluid was clear with small fibrin clots: these smeared on a slide showed microscopically polymorphonuclear cells containing Gram-negative cocci, resembling meningococci. Inoculation of this material on blood-agar yielded colonies of (1) Meningococci, (2) Gram-positive staphylococci, (3) Streptococci resembling Pneumococci.

*Specimen 3.* (Which yielded strain 4*M*.) Lumbar puncture fluid from a child aged  $4\frac{1}{2}$  years, under the care of Dr W. Carr at the Victoria Hospital. The child died of meningitis after about three weeks' illness: no post-mortem. The fluid was examined by Dr W. E. Marshall. A single colony appeared on the cultures which resembled a meningococcus colony, and consisted of Gram-negative cocci.

*Specimen 4.* (*M*. 8.) Cerebro-spinal fluid obtained by lumbar puncture after an indefinite illness of some weeks' duration from a child aged 4 months under the care of Dr W. Carr, at the Victoria Hospital. The fluid was clear and contained some leucocytes, mostly polymorphonuclear, and streptococci. Cultures yielded a Gram-negative small oval bacillus which liquefied gelatin. Post-mortem no macroscopic meningitis.

*Specimen 5.* (*M*. 10.) Post-mortem material from the meninges of a child aged 3 months under the care of Dr Montague Murray at the Victoria Hospital, who kindly allowed me to make cultures. There was a history of only 26 hours' illness. Post-mortem tough yellowish exudation was found under the pia mater. A smear showed a pneumococcus-like diplococcus. Cultures from the meninges gave almost pure Gram-positive lancet-shaped cocci. The pneumococci still showed capsules in agar culture after the second generation.

*Specimen 6.* (*M*. 11.) Cerebro-spinal fluid removed by lumbar puncture from a child aged 9 months with symptoms of posterior basic meningitis, under the care of Dr W. Carr at the Victoria Hospital. A smear of the deposit in the fluid showed many polymorphonuclear leucocytes and a few lymphocytes and large mononuclears,



and cocci which morphologically resembled meningococci. Culturally (1) meningococci found but not isolated, (2) Gram-positive cocci, and (3) a Gram-negative short bacillus.

*Specimen 7.* (*M.* 27.) Cerebro-spinal fluid, from a case of meningitis, which was sent by post and was almost 48 hours on the journey. Broken up cells and no cocci were seen microscopically. Only Gram-positive streptococci and staphylococci were isolated, probably contaminations.

*Specimen 8.* (*M.* 34.) Cerebro-spinal fluid which was about 20 hours in the post, from a typical case of cerebro-spinal meningitis on 4th day of disease. The fluid was watery in appearance and gave a very small deposit on centrifugalising. Microscopically many polymorphonuclears were seen but no cocci.

In the cultures only Gram-positive staphylococci appeared.

*Specimen 9.* (*M.* 33.) Lumbar puncture fluid from a typical case of cerebro-spinal meningitis at the 6th day of the disease. The specimen was about 20 hours in the post; no leucocytes nor cocci were seen after centrifugalising. Cultures yielded a Gram-positive staphylococcus and a bacillus resembling the *Bacillus coryzae segmentosus*.

*Specimen 10.* (Yielding strain *M.* 38.) Lumbar puncture fluid from a child aged 5 suffering from meningitis, which was about 16 hours in post, had a deposit of thick pus which was composed chiefly of polymorphonuclear leucocytes some of which contained a few (1 to 3) large Gram-negative cocci of typical appearance. Meningococci grew freely in cultures and one or two colonies of a *Staphylococcus albus* appeared.

The strains of meningococcus XII, XVII, XVIII, XIX, XXIX, and *M.* 38, when grown on blood agar, formed small faintly milky and raised colonies, which after two or three generations grew on ordinary agar at 37° C. as translucent, very slightly whitish colonies, which were confluent when much crowded, and were slightly brownish or amber as seen by transmitted light. Under a 1-inch objective the colonies were seen to be very finely granular or quite smooth. The growth was rather sticky and slimy. The earlier generations died if not sub-cultured every three days, but later generations if protected from drying by tight plugging of the tube, sometimes lived for one month at 37° C. At room temperature sub-culture was never successful after three days. In ordinary broth at 37° C. growth took place (though very slowly when first isolated), a general turbidity being caused, and later a whitish muddy deposit.

When 1% of various carbohydrates was added to peptone water and broth (75% of the former, 25% of the latter) acid was formed in three days from maltose and glucose, and later from galactose and laevulose in the case of XII, XVII, XVIII and XIX; but XXIX, only produced acid from glucose after prolonged artificial culture and not constantly from laevulose. XXIX never formed acid from galactose. None of



these strains produced acid from cane sugar or lactose. The medium became turbid and a whitish ring, adherent to the glass, formed at the upper level of the liquid in seven days.

In no instance was the result altered by adding ascitic fluid or serum to the sugar-broth, though the change was sometimes hastened.

These strains did not grow on gelatin at 22° C., but on one occasion when the incubator was raised to 24° C. for a few hours, a very slow growth of XVIII began and continued at 20° C., liquefying the gelatin at the surface. The purity of this culture was repeatedly proved by sub-culture and microscopic examination. Accordingly I made cultures on gelatin of four (XII, XXIX, XVIII, and XIX) of the strains of meningococcus, strain 4*M.* and several strains of *M. catarrhalis* obtained from the nose, and incubated them at 37° C.; after 10 days the gelatin containing strains XVIII, XXIX and XII remained liquid, but was of a thick syrupy consistency, on cooling to 15° C., whereas those tubes containing XIX, 4*M.* and the different strains of *M. catarrhalis* became quite solid.

It, therefore, appears that some difference exists among strains of meningococcus, in the power of producing acid from sugars and in that of liquefying gelatin.

Those strains isolated from typical posterior basic cases showed the most aberrant characters.

The strain 4*M.*, which was found in pure culture and a single colony isolated from the cerebro-spinal fluid of a case of meningitis, is particularly interesting, in that it agreed in some particulars with the meningococcus, but was certainly not a member of this species, and also because Gram-negative intracellular cocci of very similar shape and appearance to meningococci were seen by Dr W. E. Marshall in polymorphonuclear leucocytes in a smear of the same specimen of cerebro-spinal fluid. Of course these may have been meningococci which did not grow in the culture tubes. Only one colony had grown on the blood agar at the end of 48 hours, and this was indistinguishable from a colony of meningococci in appearance. On sub-culture, however, it grew well on agar, but always discretely, and the colonies were whiter than those of meningococcus. It grew in broth rendering the medium uniformly turbid, and the colonies on agar were not coarsely granular and were moist and rather sticky. An emulsion was easily made and did not agglutinate spontaneously. In these respects 4*M.* resembled the meningococcus very closely, but it grew on gelatin at 22° C. without liquefaction; it failed to produce acid from glucose,

maltose or galactose, and an emulsion was not agglutinated by meningococcus serum in dilutions of 1—2 and 1—10. Moreover in a film from the original colony the cocci were rather larger and stained better with methylene blue than it is usual for meningococci to do. It is, therefore, clear that this organism is not the meningococcus. It resembles *Micrococcus cinereus* of v. Lingelsheim in most particulars. (See my paper on *Micrococcus catarrhalis* &c. in this *Journal*, Jan. 1907, Table III shewing cultural differences.)

I made four experiments with regard to the power which the meningococcus has to withstand drying.

*Experiment 1.* Impressions of blood agar colonies of XXIX and XVIII on coverslips were put into dry sterile tubes and left at room temperature for 2 days, and then transferred to broth and incubated at 37° C. The meningococcus could not be recovered from them.

*Experiment 2.* Portions of broth cultures of strains XVIII and XIX were dried in sterile Petri dishes (*a*) mixed with sterile sand, (*b*) mixed with sterile violet powder, (*c*) without admixture. The dishes were then placed in an air-pump receiver over strong sulphuric acid and the air exhausted. Samples taken at the end of 24 hours did not contain living meningococci.

*Experiment 3.* Some of a broth culture of strain XXIX was dried on strips of sterile blotting paper or on sand, over sulphuric acid in the receiver of an air-pump; after three hours the sand looked dry but was still damp; from a sample put into broth and incubated at 37° C. meningococci grew. From a strip of blotting paper taken at the same time and put into broth at 37° C. no meningococci grew. After 24 hours and complete drying of the sand no meningococci could be recovered.

*Experiment 4.* A broth culture of strain XII poured on dry sterile garden soil in a Petri dish and left at room temperature yielded no meningococci after 20 hours.

To test resistance to cold, agar cultures of four strains of the meningococcus (XII, XVII, XVIII, XIX) were put in the cold room at 1° C. They could be sub-cultured after 72 hours.

In order to further test the susceptibility of this organism to cold, the cerebro-spinal fluid of specimen 10 was placed in the cold room at 4° C., and successful cultures were made after 48 hours, but not after 72 hours. Before this specimen reached me 15 hours had already elapsed since its removal from the body.

This high degree of viability is in marked contrast to that observed in the case of some strains such as those contained in specimens 7 and 8, which performed the same journey but could not be grown when received though similar care and media were used. It seems probable therefore that the rapid death of meningococci in lumbar puncture fluid, which has often been observed, is not due to cold but to some other

cause, such as the presence of other bacteria or of harmful substances in the cerebro-spinal fluid as suggested by Flexner (1906).

### *Agglutination Experiments.*

To prepare an agglutinating serum from a rabbit it is necessary to give large and frequent doses of meningococci intravenously for a considerable time, and the serum appears to quickly lose its agglutinating properties if the injections are discontinued.

Agglutinating serum was obtained for me by Dr W. E. Marshall by injecting large quantities of meningococci intravenously into three rabbits; the doses chiefly used were from 2—4 agar tubes, and the injections had to be given for 4—5 weeks before the serum showed much agglutinating power. I used the microscopic method of examination for agglutination.

Rabbit I was injected intravenously with 2-day agar cultures of meningococcus XII, emulsified in a 0.9 % sodium chloride solution and killed at 65° C. for 30 minutes.

TABLE I.

### *Agglutination with serum from Rabbit I.*

		1/2	1/10	1/25	1/50	1/100	1/200	1/500	1/1000	Normal 1/2
March 30	Microscopic									
	XII & XVIII 1½ hrs.	-		-	-					
	Macroscopic									
	XII 24 hrs.			-	-	-				
April 26	Microscopic									
	XII 1 hr.	+	+	sl.		-				-
May 1	Microscopic									
	XII 1 hr.		+++	++	+	++	+	sl.	+	-
	XIX „		+++	++	+	++	++	+	-	
	XVIII „	+++	+++	++	+	+	+	+		
	XVII „	+	++	++	+	+	sl.			
	XXIX „	+	-	sl.	sl.	-	-			
May 19	XII „	+	sl.							
	XXIX „			+	sl.					
				1/20						1/2000
May 25	XII „	+	+	++		+	++		+	-
	XVIII „	+++	+++	+++	+++	+++	+++			
	XXIX „	+++	+++	++	++					
	4M. „	-	-	-						

- March 9th. 4 agar tubes injected intravenously.  
 „ 16th. 7 agar tubes injected intravenously.  
 „ 30th. Bled : the serum did not agglutinate meningococcus XII nor XVII in a dilution of 1—2.
- April 6th. 4 agar tubes injected intravenously.  
 „ 12th. 3 agar tubes injected intravenously  
 „ 17th.  $3\frac{1}{2}$  agar tubes injected intravenously  
 „ 21st. 4 agar tubes injected intravenously  
 „ 24th. Bled : agglutination of meningococci in 1—2 and 1—10 dilutions.
- May 1st. Bled : agglutination good 1—200 to 1—500.  
 „ 9th. 3 agar tubes injected.  
 „ 18th. 3 agar tubes injected.  
       Bled.  
 „ 23rd. Bled.

Rabbit II. Injected intravenously with 2-days' old agar cultures, killed at 65° C. for 30 minutes.

- March 9th. 4 agar tubes injected.  
 „ 16th. 7 agar tubes injected.  
 April 3rd. Bled : no agglutination.  
 „ 6th. 4 agar tubes injected.  
 „ 12th. 3 agar tubes injected.  
 „ 17th.  $3\frac{1}{2}$  agar tubes injected.  
 „ 21st. 4 agar tubes injected.  
 „ 24th. Bled : agglutination 1—10.

TABLE II.

*Agglutination with serum of Rabbit II.*

	Microscopic	1/2	1/10	1/25	1/50
April 3	XII 1 hr.	—		—	—
	XVII „	—		—	—
April 24	XII	+	+		sl.
	XII with normal serum from rabbit.	—	—		

This rabbit shows the difficulty sometimes encountered in obtaining an agglutinating serum.

Rabbit III. Injected with agar cultures of meningococcus XXIX killed at 60° C.

- April 9th.  $3\frac{1}{2}$  agar tubes of 48 hours' growth injected.  
 „ 12th. 3 agar tubes of 48 hours' growth injected.  
 „ 17th. 4 agar tubes of 3 days' growth injected.  
 „ 24th. Bled.  
 „ 30th. 3 tubes of 48 hours' growth injected.  
 May 8th. 3 tubes of 24 hours' growth injected.  
 „ 18th. 3 tubes of 24 hours' growth injected.  
       Bled.

TABLE III.

*Agglutination with serum of Rabbit III.*

Dilution	1/2	1/10	1/50	1/100	1/200	1/1000	1/2000
9 April 1906							
XXIX	-	-	-				
26 April 1906		++	++	++	+		
19 May 1906			++	++	++	+	

One sample of cerebro-spinal fluid which I examined, and which was drawn by lumbar puncture from a child who had been ill about 13 days, agglutinated the corresponding strain (XXIX) in dilution of 1—5, and other strains (XVII and XVIII) in dilutions of 1—100 and 1—50 respectively.

TABLE IV.

*Agglutination with cerebro-spinal fluid from the same case from which Strain XXIX was isolated.*

Dilution	1/2	1/20	1/50	1/100
XXIX	+	-	-	-
XVII	+	+++	++	+
XVIII	+	++	+	-

The serum of rabbits repeatedly injected with cultures of *M. catarrhalis* did not agglutinate the *meningococcus*, and could not be tested on *M. catarrhalis* on account of the constant spontaneous agglutination of this organism.

The agglutinating power of *M. catarrhalis* serum has been tried by v. Lingelsheim (1906). He found that it never agglutinated the *meningococcus* in higher dilution than 1—50 macroscopically.

### *Conclusions.*

1. Gram-negative cocci obtained from the cerebro-spinal fluid are not always true meningococci, even in cases of meningitis.

2. Slight differences between different races of meningococci occur, especially as regards their growth and activity in sugar media and on gelatin.

3. The meningococcus is not easily killed by cold, therefore its rapid death in lumbar puncture fluid and post-mortem material must be due to some other cause.

4. The means by which the meningococcus is carried from the diseased to the healthy can hardly be such as to involve drying.



I am indebted to the late Dr A. Macfadyen for his help and suggestions, also to Dr A. E. Boycott for his advice and assistance, and to Dr W. E. Marshall for his help in connection with the animal inoculations. I have also to thank Dr Morley Fletcher, Dr Montague Murray, Dr Walter Carr, and Dr M. Macdonald for supplying me with material.

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# ON THE ABSORPTION OF ANTIBODIES FROM THE SUBCUTANEOUS TISSUES AND PERITONEAL CAVITY.

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*(Five Figures.)*

LITTLE attention has hitherto been paid to the rate with which slowly diffusing substances, such as the antibodies in general, are taken into the circulation from the peritoneal cavity or the subcutaneous tissues. The question, however, is of immediate clinical as well as scientific importance. For, if any considerable time elapse after subcutaneous injection before such a body is fully absorbed into the blood, then in urgent cases of disease we lose valuable time by injecting hypodermically the appropriate antisera. The following experiments were undertaken for the purpose of determining the rate at which such absorption occurs. They show that it is exceedingly slow. After intraperitoneal injection the antibody in the blood does not reach its maximum until 25—30 hours later, and after subcutaneous injection the interval is from 2 to 3 days.

The experiments fall into three groups according as the injections were made intravenously, intraperitoneally or subcutaneously. The antibodies used were: 1. coli-agglutinin, derived from goats immunised to the *B. coli*; 2. antitetanolyisin, derived from goats immunised to the haemolytic principle in filtered tetanus-cultures; 3. diphtheria antitoxin from horse. The animals used were rabbits, goats and man. Most of the work was done by injecting rabbits with the agglutinin. The results obtained after intravenous and intraperitoneal injections with agglutinin were confirmed by injecting rabbits with the antilyisin, and the subcutaneous results were confirmed by injecting rabbits with antilyisin, goats with agglutinin, and man with diphtheria antitoxin.

The general method employed was the same in all cases. At varying intervals of time after the injection blood was withdrawn from a vein. The serum was allowed to separate and removed from the clot 18—24 hours after the venesection. (Asepsis as strict as possible was in all cases observed.) The amount of antibody in the different samples of serum was then determined.

In the agglutinin experiments this was done by the method of Madsen and Jörgensen (1902) in the following way. Each sample of serum, as it was obtained, was stored in the cold chamber ( $2^{\circ}$ — $6^{\circ}$  C.) until the last sample was ready. Then all were examined at the same time, along with some of the agglutinating serum used for the injection. For each sample of serum a series of test-tubes was prepared. Into the first tube of each series was carefully measured a comparatively large dose of the appropriate sample of serum; into the next tube a smaller dose, into the third a still smaller—and so on to the end of the series, the dose in each tube being about  $\frac{1}{5}$ ths of the dose in the tube preceding it in the series. A similar series of tubes was made of the agglutinin injected. The volume of fluid in all the tubes was then equalised by the addition of 0.9% NaCl solution, and control tubes were prepared. To every tube was then added 1.5 c.c. of a 12 hours' bouillon-culture of *B. coli*, in which further growth was checked by the addition of a little formalin (0.05% pure formaldehyde). All the tubes were then rapidly shaken, and placed in an Ostwald water bath at  $37^{\circ}$  C. After 2 hours the tubes were removed. In each series a scale of agglutination was found, varying from complete or nearly complete precipitation in the tubes with large amounts of serum to hardly perceptible clumping of the bacilli in the lowest tubes. In the series containing the agglutinin injected, a tube was selected which showed a moderate degree of agglutination; and then in each of the other series was found the tube which showed the same degree of agglutination. By this means was determined for each sample of serum the dose which produced the same amount of agglutination, *i.e.* which contained the same amount of agglutinin. Taking this amount of agglutinin as unity, the number of units contained in 1 c.c. can be readily calculated for each sample of serum examined.

With a little practice this method gives excellent results (cf. Dreyer and Jex-Blake, 1906). A similar method was employed for the estimation of the amount of antilysin—the sera being measured against a definite dose of tetanolysin acting upon the washed red blood-corpuscles of the horse. The antitoxin holding sera were examined in the manner explained below.

I. *Intravenous Injections.* The results obtained here confirm the work of other investigators, Madsen and Jørgensen (1902), Knorr (1900), Behring (1900), etc. The antibody introduced into the circulation at one point is rapidly distributed through the blood as a whole. It at once begins to disappear, the fall in the amount in the blood occurring with great rapidity at first but with increasing slowness as time goes on, until eventually it can no longer be detected. This is shown in

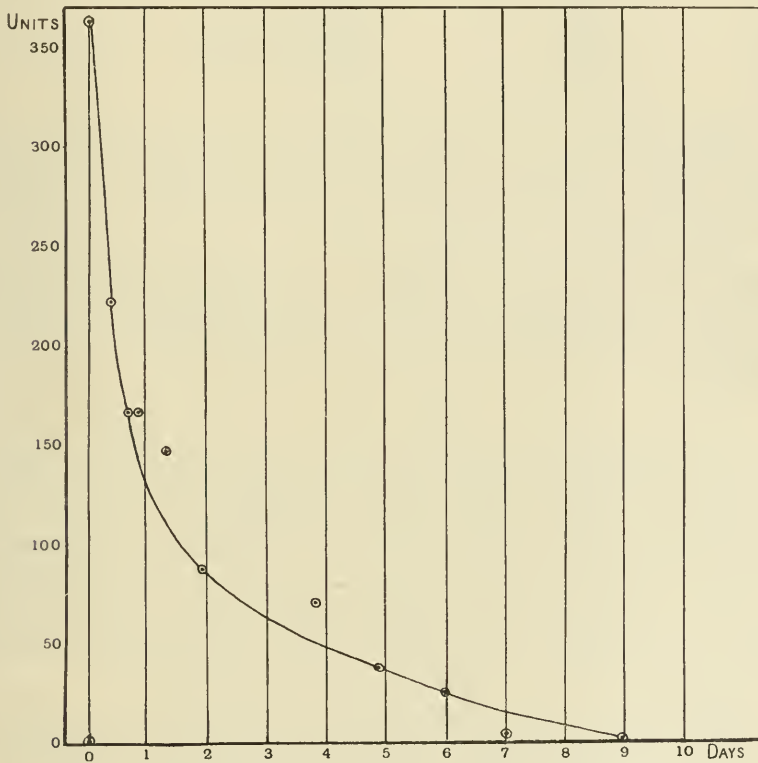


Fig. 1. Curve expressing the amount of antibody in the blood after Intravenous Injection. See this page, and Table I.

Figure 1. A control sample of blood having been first withdrawn, 15 c.c. of agglutinin were injected into the ear-vein of a fresh rabbit of 2600 grammes weight. After 15 minutes, and at each of the subsequent times stated, a small sample of blood was withdrawn. The results of the final examination are given in the following Table I. 1 c.c. of the serum before the injection produced no agglutination.

TABLE I.

The standard degree of agglutination was produced by

0.00275 c.c. of serum:	15 minutes after injection.
0.0045   "   "	9½ hours   "   "
0.006   "   "	16½   "   "   "
0.006   "   "	21   "   "   "
0.0068   "   "	1 day 8 hours after injection.
0.0115   "   "	1   "   22   "   "   "
0.014   "   "	3 days 20   "   "   "
0.026   "   "	5 days after injection.
0.0375   "   "	6   "   "   "
0.4   "   "	7   "   "   "

After 9 days, 1 c.c. serum produced only the slightest trace of agglutination. The inverse values of the doses found are used in plotting the curve in Figure 1.

Similar curves were obtained after all the intravenous injections, whether with agglutinin or antilysin. The points, with which we are concerned here, are firstly that the maximum amount of antibody is obtained in the blood at once; and, secondly, that the maximum is a high one.

II. *Intraperitoneal Injections.* The form of the curve obtained after intraperitoneal injection is different from the intravenous curve; see Figure 2. There is first a rise, corresponding to the excess of the amount absorbed from the site of injection over the amount disappearing from the blood after absorption. The rise continues for about 30 hours, and then is followed by a fall similar to that seen in the intravenous curves. The shortest time required to reach the maximum in any of the experiments was 26 hours, the longest 42 hours.

Further, the maximum reached is low. The experiments recorded in Figures 2 and 3 were all performed at the same time on rabbits of equal weights (3000, 2950, and 2900 grammes) injected with equal amounts of agglutinin. The examination of the sera was also made at the one time. The unit is the same in all, and the maxima obtained in the three cases are comparable with one another. After the intravenous injection the maximum obtained was 540 units; after intraperitoneal injection the maximum was 250, or less than one-half after subcutaneous injection the maximum was 143, or less than one-third; see Tables II. and III.

Similar results were obtained with the antilysin experiments.



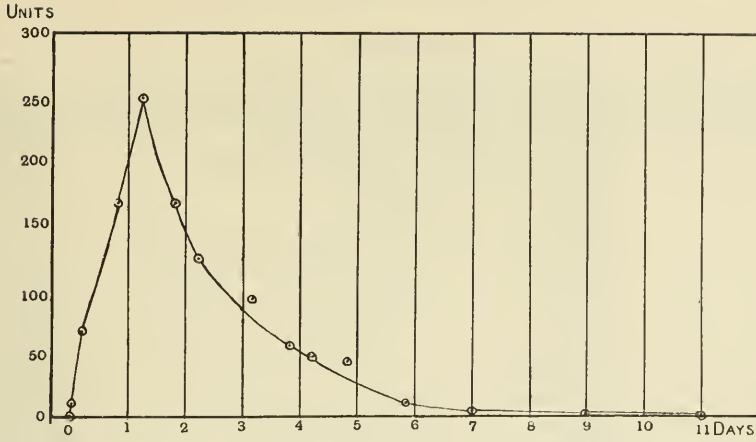


Fig. 2. Curve after Intraperitoneal Injection. See p. 208 and Table II.

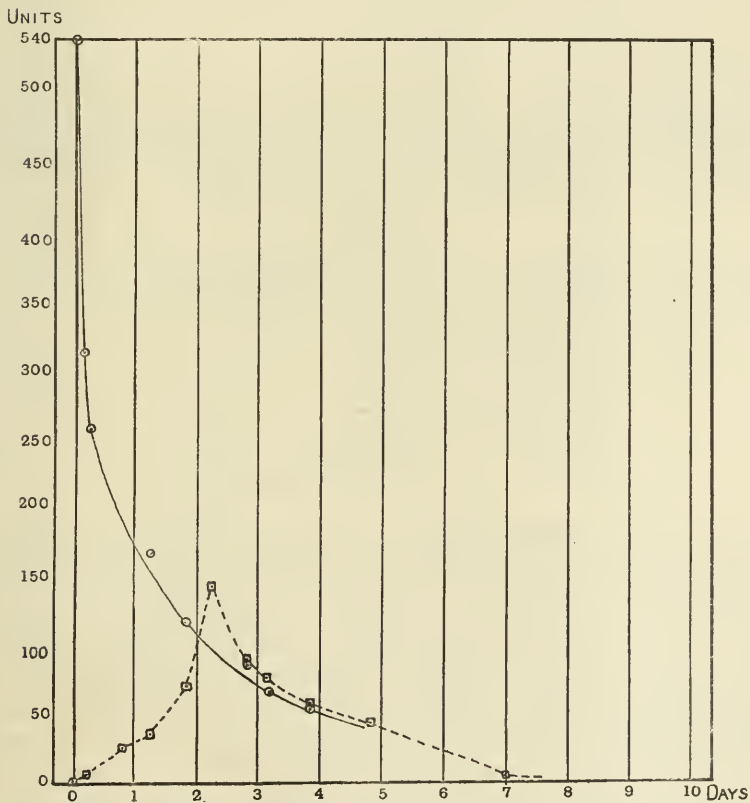


Fig. 3. The curve of Subcutaneous Injection is represented by the broken line; the curve of Intravenous Injection by the whole line. See p. 210 and Table III.

TABLE II.

Fresh rabbit, 2950 grammes, injected intraperitoneally 15 c.c. agglutinin. Before injection, 0.7 c.c. serum gave no agglutination. The standard degree of agglutination was produced by

0.08	c.c. serum:	5 minutes	after injection.			
0.015	" "	5½ hours	" "	" "	" "	" "
0.006	" "	20	" "	" "	" "	" "
0.004	" "	30	" "	" "	" "	" "
0.006	" "	1 day	20 hours	after injection.		
0.008	" "	2 days	6	" "	" "	" "
0.011	" "	3	" 4	" "	" "	" "
0.018	" "	3	" 20	" "	" "	" "
0.021	" "	4	" 6	" "	" "	" "
0.0225	" "	4	" 20	" "	" "	" "
0.085	" "	5	" 20	" "	" "	" "
0.225	" "	7 days	after injection.			
0.5	" "	9	" "	" "	" "	" "

TABLE III.

Fresh rabbit, 2900 grammes, injected subcutaneously dorsum 15 c.c. agglutinin (at the same time as the experiment of Table II.). Before injection, 0.7 c.c. serum gave the required degree of agglutination. The standard degree of agglutination was produced by

0.45	c.c. serum:	5 minutes	after injection.			
0.4	" "	3 hours	" "	" "	" "	" "
0.3	" "	5 hours	10 minutes	after injection.		
0.037	" "	20	" "	after injection.		
0.0275	" "	1 day	5 hours	after injection.		
0.014	" "	1	" 20	" "	" "	" "
0.007	" "	2 days	5	" "	" "	" "
0.0115	" "	2	" 19	" "	" "	" "
0.013	" "	3	" 3	" "	" "	" "
0.017	" "	3	" 19	" "	" "	" "
0.0225	" "	4	" 19	" "	" "	" "
0.5	" "	7 days	after injection.			

III. *Subcutaneous Injections.* The injections in the lower animals were made into the loose tissue under the skin of the back, a little to one side of the middle line. The curve of absorption is of a similar form to that obtained with intraperitoneal injections, but the delay in reaching the maximum is still more pronounced. In Figure 3 are shown two curves plotted together to illustrate the difference between subcutaneous and intravenous injection. The maximum in the subcutaneous curve is delayed until (in this case) 2 days 5 hours have elapsed. The longest interval observed in any of the lower animals, before the maximum is obtained, was 2 days 20 hours. As a rule,

it is a little over 2 days. The shortest time observed was 1 day 15 hours, in the goat whose curve is given in Figure 4. In this case it is evident from the shape of the curve, that a bleeding was not made at the time when the antibody was at its maximum, and that the true maximal period was somewhat later than that actually observed.

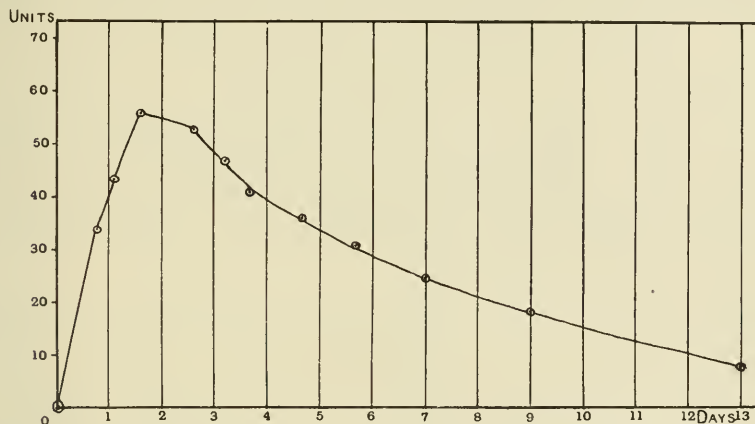


Fig. 4. Curve of Subcutaneous Injection in Goat. See this page, above, and Table IV.

TABLE IV.

Fresh goat of 13 kilos. 80 c.c. agglutinin injected subcutaneously. Before injection, 1 c.c. serum produced no agglutination. Standard agglutination was produced by

0.03 c.c. serum: 18 hours after injection.

0.023 „ „ 26 „ „ „

0.018 „ „ 1 day 15 hours after injection.

0.019 „ „ 2 days 15 „ „ „

0.021 „ „ 3 „ 4 „ „ „

0.024 „ „ 3 „ 16 „ „ „

0.028 „ „ 4 „ 16 „ „ „

0.032 „ „ 5 „ 17 „ „ „

0.04 „ „ 7 days after injection.

0.055 „ „ 9 „ „ „

0.09 „ „ 13 „ „ „

0.115 „ „ 15 „ „ „

Scattered through the literature of immunity are to be found a few incidental observances of this phenomenon of slow absorption. Thus Pfeiffer and Friedberger (1904) noted its occurrence in rabbits with cholera-serum, and Bulloch (x. 1898) in a donkey injected with diphtheria antitoxin. In nearly all of these cases the observers' attention was directed to the end of the curve, and the earlier stages

of the process were not systematically examined. The only instance I have been able to find, where the blood was examined at frequent short intervals after subcutaneous injection, is recorded by Behring (1897). It is the case of a goat injected with diphtheria antitoxin, and the slow rise in the blood is well-marked.

Although these observations are so few and unsatisfactory, they serve to confirm the experiments recorded here and to show that the very slow absorption of antibodies into the blood is the rule in the lower animals. The subcutaneous tissue, however, is so different in the case of man that it was desirable to study the absorption in the human subject. An attempt was made with antitetanolyisin, but when the sera were examined it was found that normal human serum is in itself very strongly antilytic to tetanolyisin, and no reliance could be placed on the results obtained.

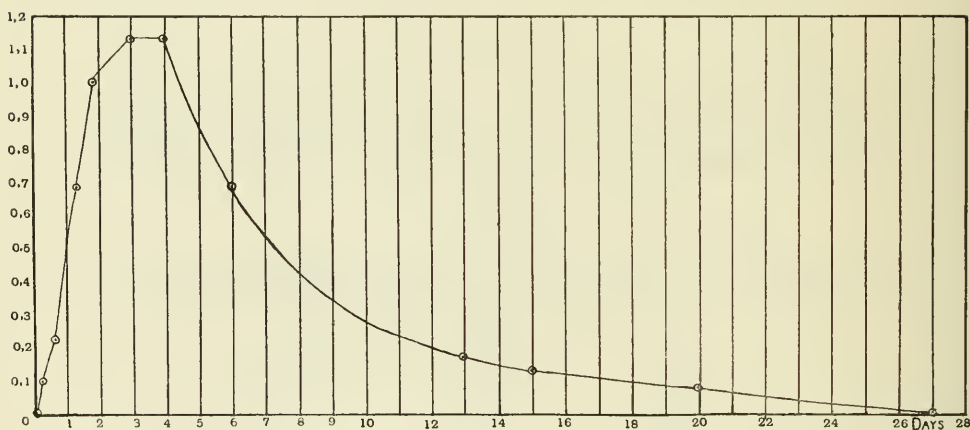


Fig. 5. Curve of Subcutaneous Injection in Man: Diphtheria Antitoxin.  
See this page, below, and Table V.

An experiment was therefore made with diphtheria antitoxin. 9000 units were injected into the subcutaneous tissue of the abdomen of a normal healthy man, weighing 72 kilos: and at successive intervals thereafter blood was withdrawn from the veins of the forearm. In 18—24 hours the serum was taken off the clot, and shortly afterwards injected together with toxin into guinea-pigs. The animals used nearly all weighed 250 grammes: in no case were they less than 240 or more than 270 grammes at the time of injection. The toxin used was the Test Toxin of the Statens Serum-Institut at Copenhagen, and was standardised in terms of the Ehrlich antitoxin unit. Of the toxin, 0.02 c.c. was

neutralised by 0·00015 c.c. of the serum injected, and this amount of serum contained 0·0675 units. The quantity of serum obtained from each bleeding, which just sufficed to neutralise 0·02 c.c. of the toxin, was determined. The amount of serum containing 0·0675 units was thus ascertained for each bleeding, and from this the number of units per 1 c.c. of serum was obtained. The results are shown in Table V.

TABLE V.

1 c.c. of the patient's serum contained :

Before injection : no demonstrable antitoxin.			
5 hours after injection : 0·1 units antitoxin.			
14	„	„	0·225 „ „
32	„	„	0·68 „ „
44	„	„	1·0 „ „
3 days	„	„	1·13 „ „
4	„	„	1·13 „ „
6	„	„	0·68 „ „
13	„	„	0·17 „ „
15	„	„	0·14 „ „
20	„	„	0·08 „ „
27	„	„	no demonstrable antitoxin.

These figures are plotted in Figure 5. The curve shows that the maximum is not reached till the end of the third day, *i.e.* 72 hours after the injection. During that period the amount of antitoxin in the blood increases steadily. It is still at the same level 24 hours later, and may have risen and fallen again in the interval. Three weeks after the injection antitoxin is still demonstrable in the blood, but it has disappeared after 4 weeks. Cf. Behring (1897), Bulloch (*x.* 1898), Bomstein (1897), Müller (1897).

The maximum content reached was only 1·13 units per 1 c.c. On the view that the amount of serum in man is  $\frac{1}{48}$ rd of the body weight (Haldane, 1900), the patient in this case contained approximately 1674 c.c. of serum. The maximum amount, then, that the serum would have contained, if all the antitoxin injected had been present in the blood at one time, is  $9000 \div 1674$ , or about  $5\frac{1}{3}$  units per 1 c.c. The amount actually obtained was less than one-fourth of this total.

The clinical bearing of these results may be briefly pointed out. It is generally admitted that in advanced cases of diphtheria, or other diseases where antisera are commonly employed, it is desirable to introduce into the system as quickly as possible as much as possible of the appropriate antiserum. Any, even the shortest, delay is to



be avoided. If we inject hypodermically we are losing time. Two or three days elapse before the injection is fully absorbed, and even then the amount actually circulating at one time is very considerably less than we may obtain by other means. It seems clearly indicated that in all urgent cases intravenous injection should be performed. It would be well to follow this up by a subcutaneous injection also, in order that the antitoxin slowly absorbed from the tissues may counteract the rapid loss after the intravenous injection. The amount in the circulation could thus be maintained at a higher level for a longer time.

The results of the subcutaneous experiments have an interest in a different connection. Ransom (1901) has shown that antitoxin injected into the subcutaneous tissues is removed by the lymphatics and not by the blood-vessels. Now, it is commonly thought that the lymph-vessels open into the connective-tissue spaces by direct communication. If this be so, it is difficult to account for the extreme slowness with which the absorption occurs. This difficulty disappears, if we suppose with Ranvier that the lymph-system is closed as the blood-vascular system is closed, and that transfusion of fluids takes place through the walls of this system. Rapidly diffusing substances, such as strychnine, will pass readily through these walls, and be absorbed with great rapidity. On the other hand, slowly diffusing bodies, such as the antibodies, will diffuse with difficulty through such membranes, and considerable time will be required for their complete absorption into the general circulation. These experiments thus lend support to the view that the lymphatic system is a closed one.

#### *Summary.*

1. Antibodies in general are absorbed very slowly from the peritoneal cavity in lower animals, and from the subcutaneous tissues in man and animals. Absorption from the latter is not complete until at least 2—3 days have elapsed.

2. The amount of antibody present at any one time in the general circulation after intraperitoneal or subcutaneous injection is very much less than the amount injected.

3. Clinically, in urgent cases of disease, to inject antibodies subcutaneously is not only to lose 2—3 days' time before the full action can be obtained but to reduce the amount of action that the dose injected can have.

4. By intravenous injection the maximum amount of action is obtained at once.

The whole of the experiments recorded in this paper were made in the Statens Serum-Institut at Copenhagen, where every possible facility was given me for the carrying out of this research. To Dr Thorvald Madsen I desire to express my thanks not only for his unvarying kindness and courtesy, but also for suggestions and assistance constantly given. I am pleased to have this opportunity of expressing my indebtedness to him.

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## THE ACID COAGULATION OF MILK.

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IT is well known that when milk is allowed to sour, the rise in acidity is not directly proportional to the increase in the number of acid-forming bacteria. There is a distinct phase in the process during which numerical increase of these organisms takes place, accompanied only by slight acid production, after which the acidity rises very rapidly to a maximum.

Two reasons have been assigned for this delay :—

(I) The necessity for a certain lapse of time, during which the organisms produce an enzyme, the function of which is the eventual formation of lactic acid from the lactose present, and,

(II) That the lactic-acid-producing bacteria do not find in milk their normal habitat, and only acquire the power to produce acid after a certain lapse of time.

The experiments here recorded are the outcome of some preliminary trials to throw some light, if possible, on the causes of the delay in acid formation, and though that end has not been attained, the results are in themselves interesting, and elucidate some of the chemical changes which take place progressively as milk sours.

It may, however, be briefly remarked that as no chemical basis for the delay has been found, it is highly probable that the power to produce acid is only developed by such organisms as *Bacillus acidilactis*, after a certain time of growth, just as may be observed in the case of certain organisms of the "coli" group, which, when inoculated into glucose-broth, often show no signs of gas production during the first 24 hours (in spite of vigorous growth), yet in the ensuing 24 hours produce gas very rapidly till the maximum evolution is reached.

It would be a perfectly reasonable supposition, and in the face of the experiments of some other observers, a very likely supposition that the lactic acid produced is at first neutralised, completely or partially, by some constituent of the milk, and so removed from the sphere of action. This, however, is not the case, as the following experiments prove:—

Definite quantities of milk, immediately after milking, were mixed with varying amounts of a solution of ordinary lactic acid of known strength, and the increase in acidity of the milk determined in each case after the addition. By the word “acidity” throughout this investigation, is to be understood that which is measured by the addition of decinormal soda to a neutral point determined by phenolphthalein. As milk itself immediately after milking shows a considerable acidity to this indicator, the increase of acidity above this number is taken to be due entirely to lactic acid, either added or produced. Determinations of acidity are best carried out in the following manner:—10 c.c. of milk are placed in each of two long Nessler glasses of 50 c.c. capacity, 10 c.c. of water added to each, and to one, 2 or 3 drops of the indicator. The contents are mixed by rotary shaking and being held side by side in one hand, N/10 NaOH is run in till the pink colour is just permanent, the blank by the side enabling this point to be determined with great exactitude.

For the experiment mentioned above a solution of N/10 lactic acid was employed, and the following results obtained:—

*Experiment I.*

10 c.c. milk required	...	...	...	1.45 c.c. $\frac{N}{10}$ NaOH	
1 c.c. lactic acid solution added to this required				0.9	„ „ (in addition)
10 c.c. milk and 1 c.c. lactic acid solution required				2.5	„ „

*Experiment II.*

10 c.c. milk required	...	...	...	1.45 c.c. $\frac{N}{10}$ NaOH	
3 c.c. lactic acid solution added to this required	...			2.9	„ „ (in addition)
10 c.c. milk and 3 c.c. lactic acid solution required				4.5	„ „

*Experiment III.*

10 c.c. lactic acid solution required	...	...	...	2.2 c.c. $\frac{N}{10}$ NaOH	
10 c.c. milk and 10 c.c. water required			...	1.75	„ „
10 c.c. milk and 10 c.c. lactic acid solution (titrated at once)	...	...	...	4.05	„ „
10 c.c. milk and 10 c.c. lactic acid solution (after 35 minutes)	...	...	...	4.05	„ „

These experiments show conclusively that, within the limits of error of such titrations, lactic acid is not neutralised (as far as phenolphthalein is concerned) either when added to milk already neutralised, or mixed with milk and the combined acidity determined, neither has time any effect on the titration.

Though from such experiments it might be concluded that lactic acid does not combine with any constituent of the milk, it actually does so, but in such a loose combination, that it can be still readily determined by caustic soda in the presence of phenolphthalein. Hammarsten<sup>1</sup> concluded that casein did not combine with lactic acid, because this acid could be completely removed from casein by triturating with water, and our own experiments detailed later, show also that the combination of casein and lactic acid, as it occurs in milk, is very susceptible to the presence of water, and is readily decomposed by it. The researches of Slyke and Hart<sup>2</sup>, and later of Laxa<sup>3</sup>, have undoubtedly proved the existence of such lactates, but the experiments of these observers are open to the objection that they cannot in themselves be taken as conclusive evidence of the existence or formation of such substances in milk itself, and for this reason, viz. that the casein employed by them had been separated from milk by more or less drastic methods of solution and precipitation, repeated again and again, and it is impossible to assert that the substance obtained eventually by such methods is identical in all its properties with the original proteid as it exists in milk. It can be scarcely doubted that the severe methods which must at present be employed in the separation and purification of proteids give rise to the formation of small quantities of other substances, which have more than once been mistaken for bodies existent in the original source.

In addition to the compound, or compounds, of casein and lactic acid, the proof of the existence of which is of comparatively recent date, there are the compounds of casein and lime salts, which have long been known. The actual constitution of these compounds is very imperfectly understood, though definite substances containing 1 mol. CaO and 2 mol. CaO have been described by Söldner<sup>4</sup>, and Lehmann<sup>5</sup> also gives the percentage of CaO in casein. Eugling<sup>6</sup> has

<sup>1</sup> Hammarsten, *Jahresb. der Thierchemie*, Vol. vii. p. 160.

<sup>2</sup> Slyke and Hart, *Amer. Chem. Journ.*, Vol. xxxiii. p. 461.

<sup>3</sup> Laxa, *Milchwirtsch. Zentralblatt*, Vol. i. p. 538.

<sup>4</sup> Söldner, *Landwirtsch. Versuchs-Stat.*, Vol. xxxv. p. 351.

<sup>5</sup> Lehmann, *Pflüger's Archiv*, Vol. lvi. p. 558.

<sup>6</sup> Eugling, *Landw. Versuchs-Stat.*, Vol. xxxi. p. 392.



asserted that the calcium is combined as calcium triphosphate, and the presence of this substance, together with CaO, has also been mentioned. Our own experiments entirely confirm the results of Eugling and others that the calcium is combined as calcium triphosphate  $\text{Ca}_3(\text{PO}_4)_2$  and not in any other form, though the nature of the compound cannot be in any way understood. If, on the other hand, CaO itself were present in the casein molecule, it might reasonably be expected that added lactic acid would neutralise this, and so be removed from the sphere of action; but as our experiments above show, this is not the case.

Our first experiment was to determine the amount of calcium compounds in combination with the casein, during the progressive stages of "souring." In attempting this, an immediate difficulty presented itself, in that a method of separating the casein without the addition of acid or any agent that might bring about chemical change, *had* to be employed.

Two ways alone seemed practicable:

- (I) Lehmann's method of filtration through porous earthenware;
- (II) Precipitation with alcohol.

Of these the former was to be preferred. Practical difficulties, however, presented themselves. Every attempt to obtain the casein by filtration through porous earthenware proved abortive or useless regarding the end in view, but a slight modification of Lehmann's original method using porous plates, succeeded admirably. The method finally used is as follows:—

An ordinary porous soup plate such as is used for drying crystals, is turned upside down, the edge being raised off the bench to allow free access of the air to the underneath surface; 20 c.c. of milk can, with care, be placed on the base of the plate without running over the edge. A saucer is placed over the milk to prevent evaporation (a trace of formalin being added to the milk beforehand to prevent further decomposition). The plate gradually absorbs everything but the fat and casein; when dry 10 c.c. of a very dilute formalin solution are run on and spread over the surface of the casein, and after replacing the cover, allowed to absorb as before. This is once repeated, by which time all soluble substances have been removed from the casein and can be seen as a ring far down the side of the plate. The layer of casein and fat is removed as completely as possible with a knife, and placed in a vacuum desiccator for 48 hours to dry. It is then extracted with dry ether in a Soxhlet extractor, and again dried in the desiccator.

The casein is thus obtained in perfectly white friable strips, though not, however, completely free from traces of fat. The average amount of this fat was estimated in several samples by the Schmidt method (solution in HCl), and allowed for in the weight of casein used in each experiment. This is not of course strictly correct, but the error is too small to appreciably affect the results.

In the casein so obtained estimations of calcium and phosphoric acid were made in the following manner:—

A weighed quantity of the casein is carefully incinerated in a platinum dish. (A curious property of the casein may be noted; as the casein becomes free from calcium salts, it swells up more and more on incineration, and when practically free from them, is difficult to keep in the dish.) The incineration is continued till an almost white ash is obtained, a result most easily brought about by moistening the char with a few drops of dilute HCl, evaporating to dryness, and then completing the incineration. The calcium salts are dissolved out with hot dilute HCl and filtered (to remove any fragments of porcelain that might be present) into a small beaker. In the solution so obtained the calcium and phosphoric acid are estimated as follows:—

The contents of the beaker are heated to boiling on a sand-bath, and 2 c.c. of a saturated solution of ammonium oxalate added. Allow to cool somewhat and add dilute ammonia till just alkaline to litmus (the calcium oxalate now precipitates). Make distinctly acid with dilute acetic acid and again heat to boiling, and allow to stand overnight. Filter through a small filter, and after incineration weigh as CaO. The filtrate is concentrated to about 10 c.c. on the water-bath and the phosphoric acid estimated by precipitation with magnesia mixture as magnesium pyrophosphate. After the first experiment, the calcium was estimated volumetrically, as on account of the small quantities dealt with, the error in the gravimetric method is rather large. In this case the oxalate is filtered off through a small toughened filter (it is not necessary to clear the beaker of oxalate), the filter is pierced, and the precipitate washed into a small flask with a fine-nosed washed bottle. About 10—15 c.c. of 1:10  $\text{H}_2\text{SO}_4$  are heated to boiling, and the beaker washed out with a few drops of this on to the filter, and the filter well washed with the remainder. The contents of the flask are heated to about 50° C. and titrated with permanganate solution (1 c.c. = 0.0025 grams calcium) set against pure sodium oxalate.

*Experiment I.*

Milk obtained under very careful conditions and inoculated at once with a large quantity of *B. acidi lactici*, this organism being chosen as it does not complicate the experiment by the production of gas.

2000 c.c. of the milk (a large amount is necessary in order that the daily withdrawals may affect the remainder as little as possible), were obtained in a large bottle, arranged with a side siphon tube so fitted that this could be washed out each day after withdrawal of milk, with sterile water.

To this milk were added 5 c.c. of a coagulated milk culture of the organism distributed in about 20 c.c. sterile water. The whole was thoroughly mixed, and kept at 65°—67° F. At intervals of 24 hours about 50 c.c. were siphoned off into a sterile flask, the contents of the bottle being well mixed just previously. Of the amount so withdrawn 20 c.c. were placed on the porous plate, 10 c.c. were used for an acidity determination, and a dilution was made for a bacterial count.

The following table shows the results obtained:—

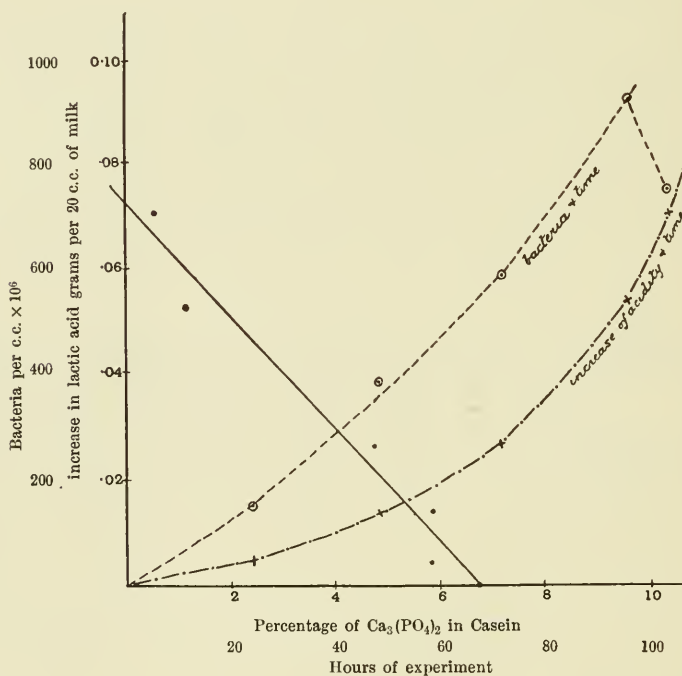
Time	Lactic acid in grams per 20 c.c.	No. of bacteria	Weight of casein (20 c.c.)	Calcium		Mg <sub>2</sub> P <sub>2</sub> O <sub>7</sub>			Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> per cent. in casein
				CaO found	Calc. as Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Found	Calc. for Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Excess	
Start	—	26 × 10 <sup>6</sup>	·5528	·0203	·0374	·0287	·0269	·0018	6·77
24 hrs.	·0046	150 × 10 <sup>6</sup>	·5091	·0161	·0297	·0256	·0213	·0043	5·83
48 „	·0142	380 × 10 <sup>6</sup>	·4878	·0155	·0286	·0242	·0205	·0037	5·86
72 „	·0257	596 × 10 <sup>6</sup>	·4943	·0127	·0234	·0195	·0168	·0027	4·73
96 „	·0545	925 × 10 <sup>6</sup>	·4915	·0026	·0048	·0146	·0034	·0112	0·97
116 „	·0714	758 × 10 <sup>6</sup>	·4204	·0018	·0033	·0042	·0023	·0019	0·79

It will be seen at once that the percentage of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> steadily decreases as the acidity increases, and if the results be plotted as a curve (see curve A) with percentages of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> as abscissae, and lactic acid in 20 c.c. as ordinates, an approximation to a straight line curve is obtained. The results are rather irregular, owing to the rather large error of experiment, but in the later experiments (as will be seen) the approximation to the straight line is much closer. The point at which the percentage of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> would become zero, is the moment of precipitation of the casein. Perfect freedom from Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> is in practice not obtainable.

From the fact that a straight line curve is obtained, we further deduce that the amount of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> present in the casein is directly

proportional to the amount of lactic acid present until the milk coagulates.

As the CaO as estimated decreases, so also does the phosphoric acid, and on calculating the amount of magnesium pyrophosphate which should be found if the CaO estimated were entirely derived from  $\text{Ca}_3(\text{PO}_4)_2$  and subtracting this from the magnesium pyrophosphate as estimated in each case, a slight excess of pyrophosphate is always present, which is naturally to be expected, seeing that the proteid itself furnishes a small amount of phosphorus which will be estimated together with that in combination with calcium.



Curve A.

As no other combination of the phosphoric acid and calcium oxide than that of calcium triphosphate will agree with the estimated quantities of these substances, and further as they decrease in the same relative proportion, we infer that the whole of the calcium in casein as it exists in milk, is combined in the form of calcium triphosphate and is gradually eliminated with increasing acidity in this form, and this form only, or if not actually present in this form, in

such a state that on separation and incineration of the proteid, this substance results.

As experiments in which bacteria are the source of the acid are subject to complications of an unknown character, a few trials were made in order to determine whether the same results could be obtained by the addition of lactic acid itself. These having shown that the action was, as far as could be seen, identical, the first experiment was repeated as follows:—

### *Experiment II.*

2000 c.c. of milk obtained as before, were mixed with 50 c.c. methylated ether, well shaken and cooled. Trial had already shown that such milk would keep at least 7 days at 65°—66° F. without any increase in acidity or the appearance of bacterial activity.

It was also determined, as a preliminary, that the action of the lactic acid is practically instantaneous, and that no further effect is produced by lapse of time.

A mixture of milk and lactic acid solution was made, one part being allowed to stand 10 minutes and the other 70 minutes before placing on the porous plate.

	Casein	Percentage of CaO
After 10 minutes	·4617	2·64
„ 70 „	·4721	2·65

The effect therefore of the acid is practically instantaneous.

An approximately N solution of lactic acid was then added in varying amounts to 100 c.c. quantities of the etherised milk, and as before the casein separated, and the calcium and phosphoric acid estimated.

The results were as follows:—

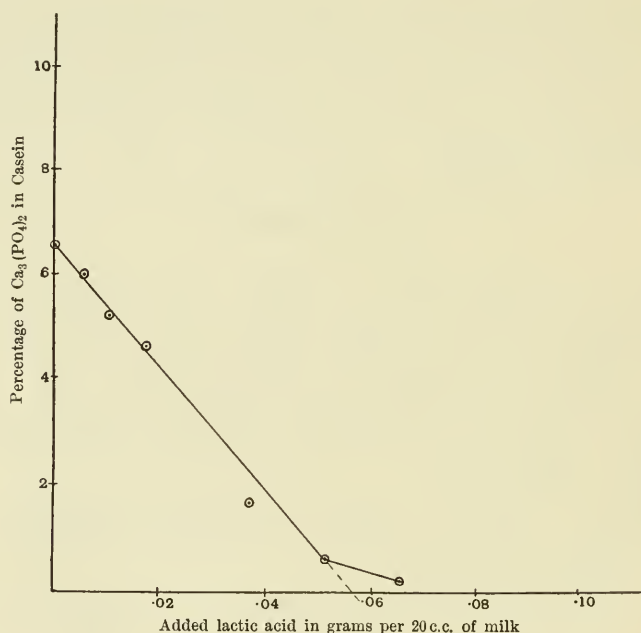
Lactic acid in grams per 20 c.c.	Casein obtained in grams (20 c.c.)	Calcium		Mg <sub>2</sub> P <sub>2</sub> O <sub>7</sub>			Percentage of Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> in casein
		CaO obtained	Calc. as Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Found	Calc. on Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Excess	
—	·534	·019	·035	·0275	·0253	·0022	6·60
·0056	·499	·017	·031	·0253	·0221	·0032	6·05
·0102	·554	·015	·029	·0241	·0208	·0033	5·20
·0170	·543	·013	·025	·0217	·0178	·0039	4·60
·0372	·525	·005	·009	·0103	·0065	·0038	1·70
·0514	·434	·001	·003	·006	·0020	·0040	0·60
·0646	·436	·0004	·001	trace	—	—	0·20

These results are plotted as curve B.



In this experiment the approximation to a straight line curve is very close.

Other experiments on the same lines gave identical results.



Curve B.

The experiments of Slyke and Hart might seem to throw a certain amount of doubt on these results, in view of the fact that they record the formation of a soluble casein lactate. If this substance be found in milk it is to be inferred that in such experiments as above recorded, this soluble lactate will pass into the porous plate, and consequently the casein obtained by this method will consist, not of the total casein of the milk, but of unchanged casein and insoluble lactate. As, however, the experiments to be recorded later throw considerable doubt on the formation of such a soluble lactate in milk under the conditions of these experiments, we are at liberty to conclude that the whole of the casein of the milk is retained by the porous plate, and that the direct proportionality between the percentage of  $\text{Ca}_3(\text{PO}_4)_2$  in the casein, and the amount of lactic acid present in the milk holds good.

It is to be remarked that a much higher percentage of calcium salts in the casein has been obtained, than has hitherto been recorded by other observers; for instance, Söldner gives 2.36%  $\text{CaO}$  in the casein

of cow's milk, and Slyke and Hart confirm this. Lehmann, using the same method as in our experiments, gives 1.45—1.75 %.

Using milk immediately it had been obtained from the cow, we have found the following results:—

- (I) 6.17 %  $\text{Ca}_3(\text{PO}_4)_2 = 3.35$  %  $\text{CaO}$ .
- (II) 6.34 %  $\text{Ca}_3(\text{PO}_4)_2 = 3.44$  %  $\text{CaO}$ .
- (III) 6.77 %  $\text{Ca}_3(\text{PO}_4)_2 = 3.66$  %  $\text{CaO}$ .
- (IV) 6.60 %  $\text{Ca}_3(\text{PO}_4)_2 = 3.58$  %  $\text{CaO}$ .

It does not seem at all likely that the compound of casein and calcium triphosphate is of a definite character, from the fact that the calcium salt is so easily eliminated by the acid, a process which seems to resemble a simple solution rather than a chemical reaction, and also, that the elimination of the triphosphate must take place equally throughout the mass of the casein, for otherwise it might be expected that the precipitation of the proteid would be similarly progressive instead of sudden, bearing in mind the fact that the acid coagulation does not take place until the elimination of the calcium triphosphate is practically complete.

We cannot confirm the statement of Slyke and Hart that “when acid is added to milk it unites with the calcium combined with the casein,” or “that on the further addition of acid the casein combines with it to form a casein salt.” The lactic acid only dissolves out calcium triphosphate from the casein, and as will be seen later the combination with lactic acid takes place concurrently with this.

As when milk is boiled a precipitation of calcium salts, especially phosphate, is supposed to occur; it seemed interesting to investigate the effect of boiling on the amount of calcium triphosphate combined with the casein.

Quantities of the same milk were used, one part being placed on the porous plate direct, and the other heated for half an hour in live steam, then rotated for a considerable time to deposit any precipitated calcium salts and the milk then placed on the porous plate. From the casein obtained in this second case  $\frac{1}{3}$ th was deducted to allow for the albumin precipitated by the heat, and of course retained by the plate.

The following are the results:—

	Casein	Percentage of CaO
Unheated milk	.819	2.72
Heated milk	.786 (corrected for albumin)	3.34

There appears therefore to be a rise in the percentage of calcium salts in the casein of heated milk.

In order to eliminate any effect due to rotation and time, the experiment was repeated. The effect (if any) of rotation on the milk was first investigated:

	Casein	Percentage of CaO
Milk standing 3 hours	·8896	2·75
Same milk rotated 3 hours	·8719	2·73

Rotation therefore has no effect on the calcium salts of the casein.

A second experiment with heated milk gave the following results:—

	Casein	Percentage of CaO
Unheated milk	·777	3·01
Heated milk	·819	3·24

It seems certain therefore that there is no elimination of calcium salts from the casein when milk is heated to the boiling point of water.

#### *The distribution of lactic acid during "souring."*

In order to determine the distribution of lactic acid when either progressively produced in, or added to milk, it is necessary to again separate the casein from the other constituents of the milk after addition of varying quantities of lactic acid, and to determine what proportion of the acid is in combination with the casein, and what proportion remains in the residue after separation of the casein.

The method employed in the former part of this investigation was clearly inapplicable. Resort was therefore had to precipitation of the casein by alcohol. Attempts to filter the coagulum produced, and to wash it with dilute alcohol, completely failed, and it became necessary to separate the precipitated casein by rotation of the liquid, removing the clear menstruum, shaking the coagulum with dilute alcohol, rotating again, and so on till no more acid was removed. The following method was finally adopted:—

40 c.c. of absolute alcohol are placed in a 100 c.c. rotation tube and 20 c.c. of the milk run in from a pipette, the tube closed, well shaken, and rotated for about 10 minutes, the clear liquid is blown off with a small wash-bottle arrangement, the coagulum mixed with 20 c.c. of 66% alcohol, shaken, rotated, and the liquid blown off as before. This washing is once repeated. The acidity of the washings after the addition of 50 c.c. water, is then found by titration with N/10 NaOH to phenolphthalein, and the casein is washed out with water into a flask and similarly titrated.

As a number of obvious objections can be raised to this method, it was necessary to closely investigate these, before using the method for the end in view.

(I) Is albumin precipitated by the alcohol?

A very careful enquiry into this showed that little, if any, albumin is precipitated with the casein, so that it may be taken that casein alone is obtained in the alcohol precipitate, the albumin appearing in the washings.

(II) As the casein lactate is undoubtedly susceptible to the presence of water, may not lactic acid be removed from the casein in quantities depending on the number of washings?

To determine this, two parallel experiments were made as above, and in one case two washings were employed and in the other three washings, the acidities of the casein and of the washings being determined as c.c. of N/10 NaOH.

	Casein	Washings
Two washings	4.0 c.c.	4.0 c.c.
Three „	4.0 „	3.9 „

It is therefore evident that the acid can be removed in a definite manner by two washings, and is not dependent on the number of washings employed.

(III) Is the proportion of acid combined with the casein and remaining in the washings dependent on the strength of alcohol employed to precipitate and wash the casein?

To determine this three quantities of 20 c.c. of the same milk were precipitated by adding to 40 c.c. absolute alcohol, but after the first rotation and separation of the clear liquid, the washings of the casein were made with, (A) absolute alcohol, (B) 66% alcohol, and (C) 50% alcohol. The caseins and washings were then titrated in the ordinary way, the alcohol strength in each of the washings being adjusted to equal volumes and the same percentage (50%) of alcohol (see *infra* IV).

The results were as follows:—

Strength of alcohol	Casein	Washings
Absolute alcohol	5 c.c.	3.8 c.c.
66 % „	4.7 „	4.0 „
50 % „	4.45 „	4.5 „

It is evident that as the amount of water used in the washing increases, more lactic acid is split off from the casein.

(IV) Has the presence of alcohol any effect on the titration? Unfortunately alcohol has a very serious effect, as will be seen from the following experiment: milk was mixed with varying quantities of alcohol and the acidity estimated in each case:—

10 c.c. milk and 20 c.c. water	1.9 c.c. $\frac{N}{10}$ NaOH
10 " " { 10 " " } { 10 " alcohol }	2.25 " "
10 " " 20 " "	2.9 " "

(V) In view of the fact that Slyke and Hart state that casein mono-lactate is soluble in 50% alcohol, it would seem that alcohol is not a suitable agent for separating the casein. The authors have not been able in any way to detect the presence of this mono-lactate, or of any lactate soluble in the strength of alcohol used; and its presence seems highly problematical for these reasons:—

(1) When the washings are neutralised, no precipitation takes place, as would be the case if a lactate of casein were present. The only proteid present in the washings in appreciable quantity is the albumin of the milk.

(2) The experiment given under heading (IV) would have given very different results. These results simply show a varying distribution of acid in a perfectly regular gradation. There is every reason therefore to suppose that the whole of the casein is precipitated by the alcohol treatment, and that the method of procedure described above is perfectly adequate for the purpose, certain careful conditions being always observed. These conditions are obvious from the above discussion. (I) Exactly the same procedure, especially as regards volume of water and alcohol present, must be maintained throughout the experiment, and (II) as strong alcohol as possible must be used for washing.

To attain these conditions the milk was titrated alone for total acidity; the caseins were washed out with water after the third rotation, and the volumes of water and alcohol in the washings were always adjusted to be the same, both in total volume and in relative proportion of alcohol to water, this being made 1:1. Absolute alcohol was used in one experiment for washings, but 66% alcohol being more convenient in practice was used in all others.

The chief effect of the presence of the alcohol in the titrations is seen from the fact that the total acidity is not always equal to the sum of the acidities of the washings and the casein.



## EXPERIMENT I.

*Absolute alcohol used for washings.*

Milk, mixed with ether immediately after milking, and used at once: varying quantities of an approximate N solution of lactic acid added with careful mixing and the casein separated as described above. Results are given in c.c. of N/10 NaOH.

Total acidity			Increase in total acidity		
Milk	Casein	Washings	Milk	Casein	Washings
2.90	1.45	1.60	—	—	—
3.68	2.16	1.74	0.78	0.71	0.14
4.81	2.78	2.12	1.91	1.33	0.52
5.59	3.61	2.29	2.69	2.16	0.69
8.92	4.95	3.97	6.02	3.50	2.37
10.97	5.21	5.61	8.07	3.76	4.01
13.07	5.86	7.41	10.17	4.41	5.81

## EXPERIMENT II.

*66 % alcohol used for washings.*

All conditions of experiment as in the above.

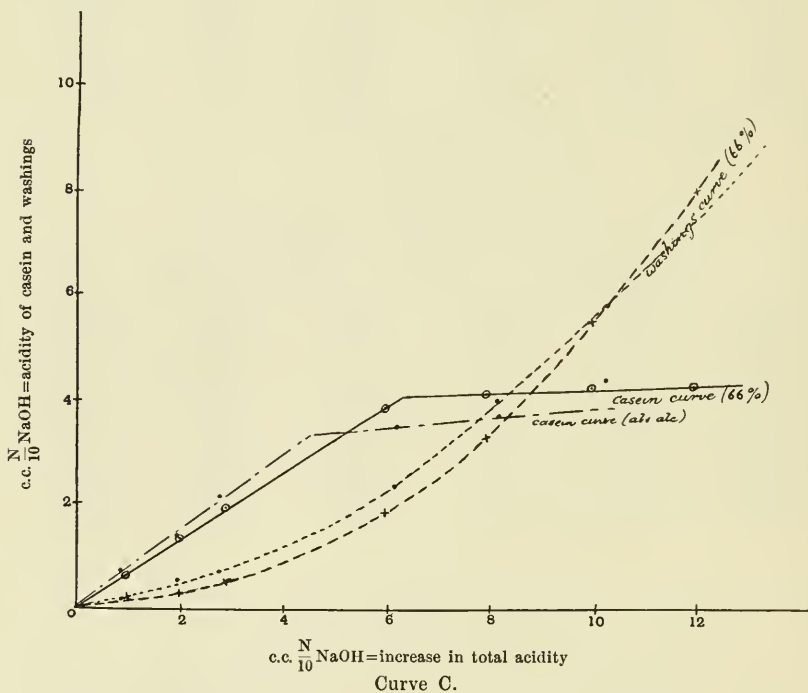
Total acidity			Increase in total acidity		
Milk	Casein	Washings	Milk	Casein	Washings
4.71	1.70	2.30	—	—	—
5.67	2.31	2.52	0.96	0.61	0.22
6.66	3.03	2.57	1.95	1.33	0.27
7.51	3.65	2.84	2.80	1.95	0.54
10.61	5.56	4.12	5.90	3.86	1.82
12.58	5.87	5.67	7.87	4.17	3.37
14.54	5.98	7.82	9.83	4.28	5.52
16.53	6.04	10.44	11.82	4.34	8.14

## EXPERIMENT III.

*66 % alcohol used for washings.*

Total acidity			Increase in total acidity		
Milk	Casein	Washings	Milk	Casein	Washings
4.39	1.39	2.79	—	—	—
5.23	2.25	2.88	0.84	0.86	0.09
5.94	2.82	3.27	1.55	1.43	0.48
6.98	3.44	3.59	2.59	2.05	0.80
10.06	5.44	4.62	5.67	4.05	1.83
12.23	4.77 (?)	6.95 (?)	7.84	3.38 (?)	4.16 (?)
14.24	5.70	8.43	9.85	4.31	5.64

The values of Experiments I and II were plotted with c.c.'s of  $N/10$  NaOH equal to rise in total acidity as abscissae, the corresponding number of c.c.'s equal to the rise in acidity of the washings and casein as ordinates. In both cases the curves obtained are closely similar, and show that up to a certain point the rise in acidity of the casein is directly proportional to the rise in total acidity: a point is then reached when an abrupt change takes place, and the casein appears to take up but little more acid. This maximum acidity, as it may be termed, is closely connected with both the precipitation of the casein and the disappearance of the calcium triphosphate from the casein, both taking place almost immediately after this point is reached.



The increase of the acidity in the washings follows a well marked curve, but no inference can be made from this, as the acidity is undoubtedly influenced by the alcohol present during the titration.

As the amount of lactic acid combined with the casein is directly proportional to the amount of lactic acid present, and the amount of calcium triphosphate combined with the casein is also directly

proportional to the amount of lactic acid present, it follows that at any moment the lactic acid combined with the casein is directly proportional to the calcium triphosphate present in the casein. Whether the lactic acid takes the place of the displaced phosphate it is impossible to say.

In conclusion, it may be inferred from these experiments that the compounds of calcium salts and of lactic acid with casein as they occur in milk, do not possess the definite compositions of those that have been formed with casein after its separation from milk, as described by Söldner for lime salts, and Slyke and Hart for lactates of casein, but that the proportions of calcium triphosphate and lactic acid in combination with the casein are at any moment before the milk coagulates the result of a sort of equilibrium between the casein and the total lactic acid present, and that at the moment of precipitation of the casein, the calcium triphosphate has been practically completely eliminated, and the combination with lactic acid has reached a maximum.

## CANINE PIROPLASMOSIS. VI.

STUDIES ON THE MORPHOLOGY AND LIFE-HISTORY OF THE  
PARASITE.

(Plates I—III and Diagrams 24—37.)

*(Continued from Vol. VI., p. 651.)*

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*(From the Pathological and Biological Laboratories, Cambridge.)**Introduction.*

IN the present paper we describe the results of further investigations on the life-history of *Piroplasma canis* in the blood of the dog. In our last paper (x. 1906)<sup>1</sup> we described and figured the movements of the parasite and the mode of multiplication in the dog's blood, and since that time we have confirmed most of our previous observations, and added further facts. The technique of these examinations was fully explained in that paper (p. 604), and it is only necessary to state here that all our later observations on living blood have been made at a temperature of 35°—40°C. The drops of blood were mounted on clean glass slides and cover-glasses kept at this temperature, and were placed as rapidly as possible on the stage of a microscope kept at a similar temperature in a Nuttall's thermostat and examined under a  $\frac{1}{12}$ th oil immersion lens. In fact we have endeavoured to make our

<sup>1</sup> Nuttall, G. H. F., and Graham-Smith, G. S. (x. 1906), Canine Piroplasmosis. V. Further studies on the structure and biology of the parasite. (Plates XI—XIII, Diagrams 1—23), *Journ. of Hygiene*, vol. vi. pp. 586—651. The bibliography is given in this paper.

observations in such a manner that the blood should be altered as little as possible.

Our examinations of the living blood have been made at all stages of the disease, at all times of the day between 9 a.m. and midnight, and have occupied more than 550 hours. Whenever necessary individual parasites have been continuously kept under observation for long periods, even up to 3 hours or more, especially in the case of forms on which our observations had previously been scanty. We, therefore, feel that we are justified in considering that if any other methods of multiplication occur than those which we are about to describe, they must be extremely rare.

Whenever fresh preparations were being examined thin smears were also made on clean glass slides and fixed in various ways. Aided by the thorough knowledge which we had gained of the various changes in shape which the living parasites undergo during the process of multiplication we have finally been able, by the examination of stained preparations, to demonstrate the accompanying nuclear changes.

In the following pages we describe (*A*) the appearance of parasites in unstained preparations; (*B*) the mode of multiplication of the parasite and the fate of various forms as observed in the living blood; (*C*) the accompanying nuclear changes as ascertained by the study of stained preparations; (*D*) the complete cycle of development within the blood.

In an appendix we give certain observations on the effect of heat on blood corpuscles.

#### (A) The appearance of parasites in fresh unstained preparations.

In our last paper (1906, pp. 605—609) we described the various appearances seen in fresh preparations of normal dog's blood with special reference to those which are likely to lead to errors in making observations on infected blood. In another place (pp. 635—639) we gave at considerable length our reasons for considering that during certain stages the parasites entered the corpuscles and divided within them. Our further observations have not led us to alter our views on this point. The means of differentiating between intra-corpuscular and epi-corpuscular parasites swimming over the surface of normal corpuscles were given on p. 613. In order to present these differences more clearly we now reproduce three sketches illustrating the appearances



of an intra-corpuseular parasite, when in proper focus, and when not completely in focus, and of a parasite lying on the surface of a normal corpuscle.

Plate I, Fig. 2*b* represents a parasite lying within a red blood corpuscle when properly in focus. It is seen that the edge of the corpuscle is darker than the central portion, and that it is surrounded by a light zone. The parasite appears as a lighter clearly defined body with a dark contour.

Plate I, Fig. 2*c* represents the same corpuscle and parasite when not yet brought into proper focus. In this case the dark and light areas previously noticed are reversed. The corpuscle is surrounded by a dark zone. The corpuscle itself is much lighter in colour and possesses an almost transparent margin. The body of the parasite, especially the marginal third, is darker than the corpuscle and it is surrounded by a light zone.

Plate I, Fig. 2*a* represents a free pyriform parasite lying on a normal corpuscle. In this case as in Fig. 2*b* the corpuscle is a dark body with a darker margin surrounded by a light zone. The parasite also appears as a dark body with a darker margin surrounded by a broad light zone. The latter varies in breadth in different cases, and appears to be due to the attenuation of the substance of the corpuscle owing to the pressure of the parasite upon it.

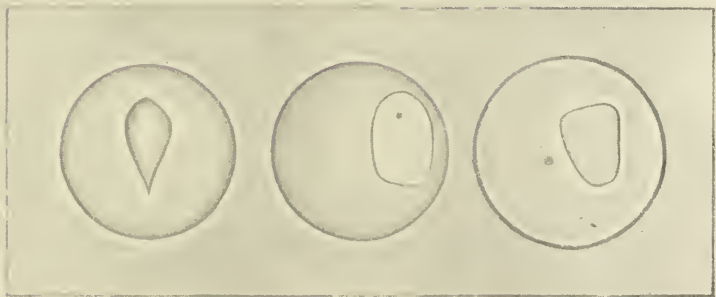
Plate II, Fig. 50 represents the appearance of a parasite lying on a corpuscle as seen in a stained preparation.

*Points already sufficiently dealt with in our last paper.*

In our last paper (1906) we fully described with the help of illustrations many of the phenomena which can be observed in the blood of infected dogs. With few exceptions all these changes have been repeatedly observed in our later experiments, and in many instances we find that we have nothing to add to our previous description. The latter include: (*a*) observations on single intra-corpuseular parasites which did not at any time show very marked pseudopodia (pp. 613—616), (*b*) observations on single parasites showing well marked pseudopodia which finally come to rest in a rounded condition (pp. 616—617), (*c*) observations relating to the movements within the corpuscles of various forms of parasites (pp. 618—620), (*h*) observations on the behaviour of certain examples of free pyriform parasites (pp. 625—631), (*k*) observations on the action of leucocytes in the blood of infected

234

Fig. 1 (11—37).



a

b

c

Fig. 2 (a—c).



dogs (p. 632), (*l*) observations made at night on the living blood (p. 633), (*m*) observations on living blood some hours after the preparation of the specimens (p. 633), (*n*) observations on the living blood within the last few hours of life (p. 634).

In fact, in our later observations we merely supplement those we had already made, except in regard to certain forms illustrated in Diagr. 21, Figs. 3, 4 and 5 of our last paper (p. 627). In this case we were mistaken in our interpretation of the bodies which we saw. These are not parasites, but degeneration products of the red blood corpuscles. Further observations on this point are given in the appendix to the present paper.

**(B) The mode of multiplication of *Piroplasma canis* and the fate of various forms as observed in the living blood.**

(1) *The entry of parasites into red blood corpuscles.* Very numerous observations have now been made on the entry of the parasites into red blood corpuscles and we are able to confirm our previous statement (p. 631) that only the pyriform or long parasites enter the corpuscles, never the round forms. The events leading up to the invasion of a normal red blood corpuscle invariably occur in the following order:

Fully mature pyriform parasites, two, four or more, escape from an infected corpuscle and moving with considerable rapidity, sometimes after short intervals of quiescence, approach other corpuscles. When one of these parasites is about to enter a normal corpuscle it generally approaches it with its blunt extremity foremost and rapidly indents its surface. Then violent movement of the thin end of the parasite occurs, and the side of the corpuscle becomes greatly distorted, and it may be caused to oscillate or even be moved from its original position. Gradually the parasite sinks more deeply into the corpuscle and finally disappears within it, when the movements of the corpuscle cease and it resumes its rounded shape. At this time it is generally difficult to define the parasite within the corpuscle, which becomes distinctly darker in colour. Gradually, however, the parasite becomes more visible, and its shape changes from pyriform to oval.

Sometimes, (see Diagr. 33<sup>1</sup>, Fig. A, p. 248), these events take place

<sup>1</sup> The Diagrams in our previous paper were numbered 1—23, and, to avoid confusion, we have numbered the Diagrams accompanying this paper 24—37.

with considerable rapidity and with comparatively little distortion of the invaded corpuscle.

Although we have continually borne in mind the possibility of a parasite entering an already infected corpuscle or of two parasites simultaneously entering a normal corpuscle, and have made especially careful and prolonged observations, whenever such events seemed probable, we have never seen either one or the other.

The entry of two parasites into a corpuscle either simultaneously or at different times, must therefore be extremely rare, under the conditions of observations on the slide where the corpuscles are spread out in a thin layer. We cannot, however, on this account be certain that these events do not occur in the body especially in the last stages of the disease, when a considerable proportion of the corpuscles are found to be infected.

(2) *The behaviour of parasites after their entry into normal red blood corpuscles.* Shortly after a pyriform parasite enters a normal corpuscle it changes its shape and becomes rounded and remains quiescent for a variable period (Diagr. 33, Figs. A and B). Most commonly it then grows in size, becomes amoeboid and finally divides into two pyriform parasites (Diagr. 24). More rarely, however, the parasite, while still small, apparently divides into two small rounded parasites, each of which subsequently behaves like a single parasite. The process of multiplication by which a single small rounded parasite becomes converted into two pyriform parasites will therefore be first described.

*The formation of two pyriform parasites from a single small round parasite.* The small round parasite after a period of quiescence gradually enlarges and shows slight changes in shape. After further growth it becomes actively amoeboid throwing out one or more blunt pseudopodia or delicate processes which are constantly changing their position and shape. Frequently a pseudopodium is retracted and another thrown out (Diagr. 24, Figs. 16—26). The movements of the parasites during this phase have been fully illustrated in our previous paper (1906, pp. 598, 613—619, living examples, Diagr. 14 and 15, and fixed preparations, Diagr. 9 and Plate XI, Figs. 3—9), and need not be further described. After a variable period of time the activity becomes greatly diminished and the parasite assumes a more or less rounded shape without any marked pseudopodia (Diagr. 24, Fig. 30; Diagr. 25, Fig. 21). After a longer or shorter time two minute rounded processes are protruded from two closely situated points on the circumference of the parasite (Diagr. 24, Fig. 30; Diagr. 25, Fig. 23).



Simultaneously the two processes gradually enlarge, particularly at their distal extremities, and become more or less pear-shaped, being attached to the main body of the parasite by relatively narrow necks (Diagr. 24. 32; 25. 30). As the protoplasm of the parasite flows into these processes a time is reached when each of the two processes and the remains of the body of the parasite are of approximately equal size, and the whole parasite has a trefoil appearance (Diagr. 24. 33; 25. 35).

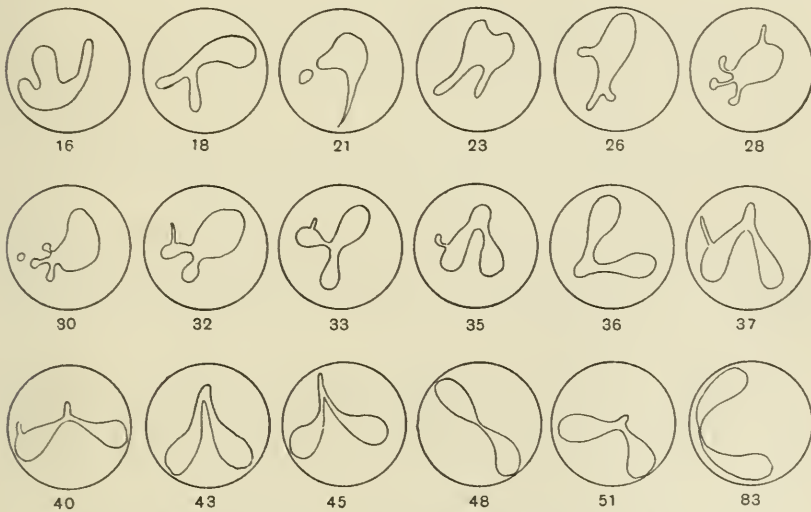


Diagram 24 representing the changes observed during the development of a single amoeboid parasite into two pyriform parasites<sup>1</sup>.

After this the simultaneous enlargement of the processes at the expense of the body of the parasite still continues and the latter becomes smaller and smaller, but in the majority of cases still retains its rounded form until it appears as a minute rounded mass to which the processes now converted into almost mature pyriform parasites are attached (Diagr. 24. 35—36; 25. 38). Ultimately this mass completely disappears and the two processes alone remain as two mature pyriform parasites, occasionally joined by a thin strand (Diagr. 24. 37—83; 25. 45).

In the accompanying Diagrams (24 to 26), drawn from typical examples of living parasites, the gradual development of two pyriform

<sup>1</sup> The numbers under the figures indicate the number of minutes which had elapsed after the preparation of the specimen.

parasites from single amoeboid forms can be more easily followed than described.

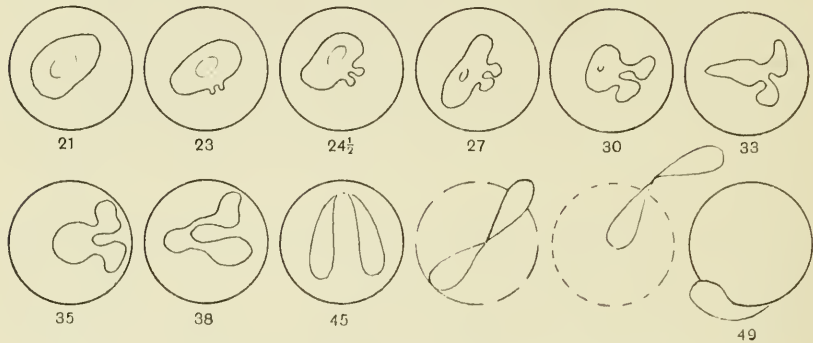


Diagram 25 representing the changes observed during the development of a single rounded parasite into two pyriform parasites.

The complete cycle has not been observed in a single specimen, but the formation of amoeboid parasites from small forms, and the formation of two pyriform parasites from amoeboid parasites have been followed on many occasions.

Although the method just described in which the main mass of the parasite gradually and symmetrically flows into the newly formed processes is by far the most common, and has been observed on very many occasions, variations are sometimes noticed, some of which are illustrated in Diagr. 26, in which many of the intermediate stages are omitted.

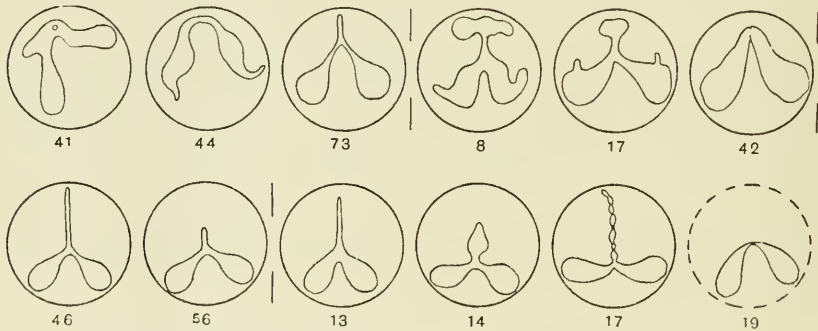


Diagram 26 representing the final stages of development of several parasites into double pyriform bodies.

The first three figures (41, 44, 73) represent the gradual transformation of a single parasite with two irregular processes up to the

stage shown in Diagr. 24. 40. In this case the main mass of the parasite was early absorbed into the process. The parasite was kept under observation for 63 minutes from an early amoeboid stage till the development of two mature pyriforms. The stages of special interest only were picked out for illustration. The next three figures (8, 17, 42) representing the transformation of a parasite from a stage corresponding to Diagr. 25. 33 into two somewhat irregular pyriform parasites, were selected from a series of sketches showing the complete development of an amoeboid parasite during 37 minutes. In the stage shown in Fig. 8, that portion which formed the main mass of the original parasite is seen to be ovoid and attached by a narrow stem to two irregular processes. After a period of 9 minutes (17) the main mass became round and the processes almost pyriform except for the fact that each showed a small projection. After a further period of 25 minutes the original parasite had completely divided into two slightly irregular pyriform parasites. In Figs. 46 and 56, the last phases of the division of a parasite are shown. In this case the main mass, instead of remaining rounded, assumed a rod-like shape, before finally disappearing. In the last four figures a somewhat similar condition is represented. The rod-like projection (13) alternately elongated and contracted before being finally absorbed. At one stage it was long and rod-like (13), at another rounded (14), and later it again became elongated and had a beaded appearance (17). Before its final disappearance it contracted and elongated several times. The last figure (19) represents the two conjoined parasites which were produced, and which ultimately escaped from the corpuscle. The broken contour line of the corpuscle is intended to indicate that the corpuscle faded before the parasites left it.

On very rare occasions we had observed the division of a single amoeboid parasite into four pyriform parasites.

*Diagram 27* (3) shows a parasite protruding two pairs of processes. Each pair exactly resembles the pair which in the method just described gives rise to two pyriform parasites. The development was followed for 5 minutes during which the processes rapidly enlarged, and the parasite almost reached the stage represented in the next figure. At this moment the corpuscle unfortunately ruptured, and development came to an end.

*Diagram 27* (2, 18, 42) shows, however, the later stages of such a method of division in which four pear-shaped processes joined to a single stem eventually separated into four pyriform parasites.

Occasionally a single small round parasite after passing through an amoeboid stage assumes the typical pyriform shape of the mature form and escapes from the corpuscle.

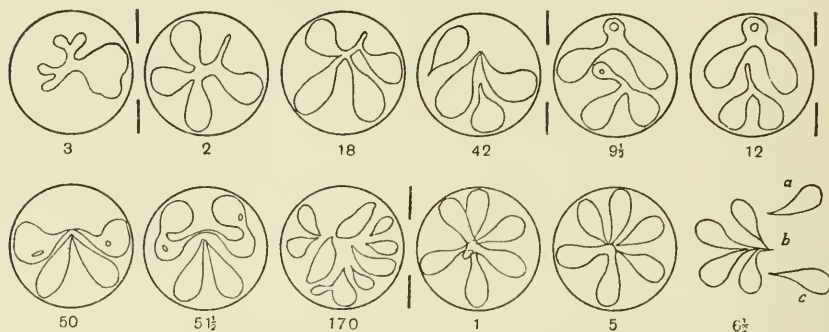


Diagram 27.

(3) *The simple division of small rounded parasites.* Red blood corpuscles containing two small rounded parasites are frequently seen both in fresh and stained preparations, but their origin cannot be easily determined. In some cases they may possibly be derived from the invasion of the corpuscle by two pyriform parasites, though we have never observed such an occurrence, but in most cases they seem to be derived from the simple division of a small rounded parasite.

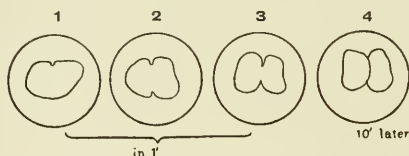


Diagram 28 representing the division of a small rounded parasite by simple division.

Although we have very carefully studied the behaviour of the small round parasites we have very seldom observed satisfactory examples of simple division. Indications of approaching division such as the formation of deep indentations on opposite sides of an elongated parasite are common (Diagr. 28. 1, 2, 3) and frequently end in the apparent division of the parasite into two smaller rounded parasites (4). Some of these appear to be cases of true division. In other cases, however, although no connection can be seen between the apparently divided portions of the parasite, on further observation the two parts again become united, showing that some delicate and

invisible bond had existed between them (Diagr. 29. 14, 15,  $15\frac{1}{2}$ ). Observations on amoeboid parasites show that outlying portions are frequently attached to the main mass by such attenuated strands that they are for a time either almost or completely invisible in living preparations (Diagr. 24. 21).

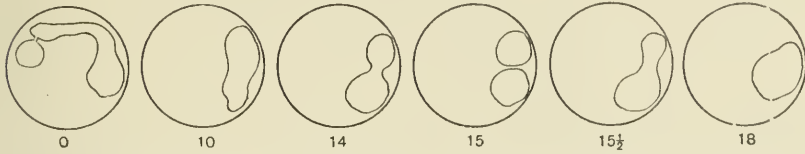


Diagram 29 representing the apparent division (Fig. 15) of a single amoeboid parasite.

Owing to the frequency of these deceptive appearances it is extremely difficult to decide whether true division has occurred or not, unless the corpuscle is kept under observation for a considerable time and the newly divided parasites definitely indicate their lack of connection by their movements.

In spite of these difficulties we are convinced that such division does occur, and this view appears to be confirmed by the examination of stained preparations.

(4) *The behaviour of double amoeboid parasites within single red blood corpuscles, and the formation of four pyriform parasites from them.* The behaviour of two amoeboid parasites within a single corpuscle has been frequently followed out. On many occasions the corpuscle ruptured and the parasites were liberated without any change in form.

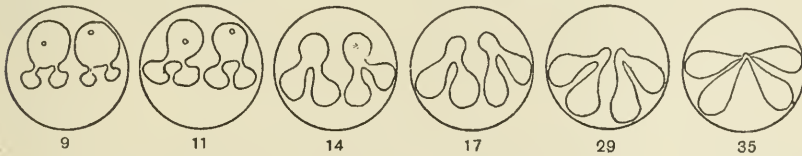


Diagram 30 representing the stages observed during the simultaneous development of two amoeboid parasites into four pyriform bodies.

On several occasions we were, however, able to observe the formation of two pyriform parasites from each of the amoeboid forms. The method is invariably the same as that which is seen in the formation of two pyriform parasites from a single amoeboid form, namely, by the protrusion of a pair of processes from each amoeboid parasite after it had become more or less inactive.

At times the changes occur simultaneously as indicated in Diagr.



30 and 31 whilst at other times one parasite is slightly in advance of the other (Diagr. 27. 9½, 12).

At other times one amoeboid parasite completes its division before the other has ceased to be actively amoeboid (Diagr. 33. 4). Under these conditions we see within an infected corpuscle two mature pyriform parasites and one large round or amoeboid form.

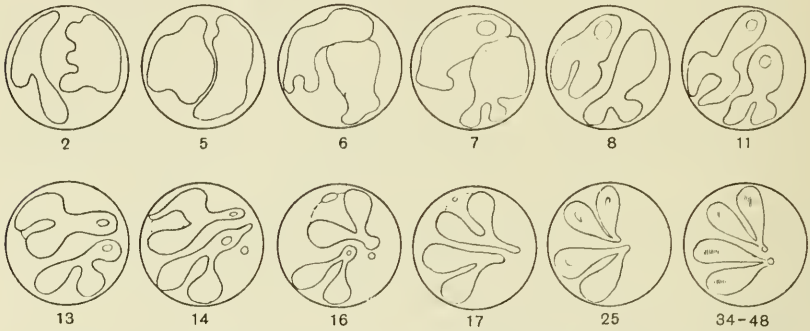


Diagram 31 representing the stages observed during the almost simultaneous development of two amoeboid parasites into four pyriform bodies.

Very rarely the single amoeboid parasite has been seen to assume a pyriform shape, thus giving rise to three pyriform parasites in one corpuscle.

(5) *The formation of several pyriform parasites in a single red blood corpuscle.* Owing to the superposition of parts of one parasite upon another, and the difficulty of following rapid movements in a corpuscle containing several parasites, it is an extremely difficult matter to clearly distinguish the formation of several pyriform parasites. Occasionally, however, the process can be followed.

*Diagram 27* (1, 5, 6½) represents the later stages in the formation of six pyriform parasites in a single corpuscle. When first noticed, immediately after the preparation had been mounted, three almost completely differentiated pairs of pyriform parasites, all in the stage illustrated in *Diagr. 24. 40* were seen, with their connecting parts overlapping. Within 5 minutes one pair had become differentiated into two completely separate pyriform parasites. Shortly afterwards the corpuscle ruptured and two free parasites and four joined to a single stem were liberated. The latter had therefore probably been formed from one amoeboid parasite as illustrated in *Diagr. 27 (2, 18, 42)*.

The subsequent behaviour of these free parasites was watched for some time. One of the single parasites swam away and after a few

minutes could not be followed, the other became round and still after 27 minutes. The four conjoined parasites appeared to attempt to swim in different directions causing the whole mass to vary slightly in position. After 21 minutes they were engulfed by a leucocyte.

Other instances of a similar kind have also been noticed showing that the same method of division is followed in the formation of multiple pyriform parasites within a single corpuscle as in the formation of a pair.

In another case two pyriform and two irregular amoeboid parasites were noticed within a corpuscle (Diagr. 27. 50—51½). The specimen was watched for some time but no further development was noticed. The preparation was however left under the microscope and examined 2 hours later. It was then seen that the irregular parasites had undergone division, and that the corpuscle then contained eight more or less regular pyriform and one irregular pyriform parasite with a process projecting from its side. No doubt could be entertained as to the identity of the corpuscle since no other infected corpuscles were near it.

(6) *Irregular pyriform parasites.* Our observations on living and stained preparations have shown that a small proportion of the parasites resulting from the division of single or double amoeboid forms are not typical pyriform parasites, but pyriforms showing certain irregularities in shape.

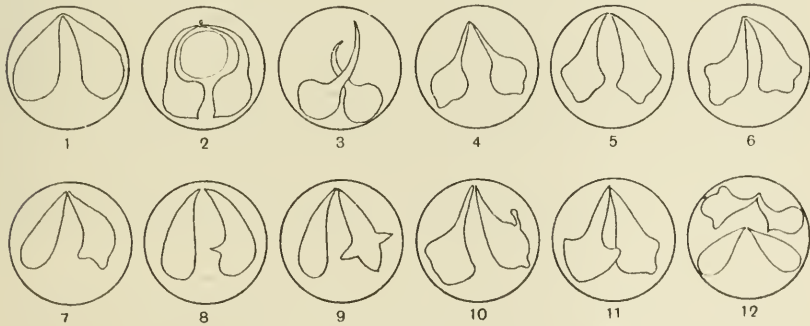


Diagram 32.

*Diagram 32* illustrates a number of these forms. Fig. 1 shows a very large but otherwise regular type, Figs. 2 and 3 types with rounded bodies and long curved tails, Fig. 4 a similar type with a straight tail, Figs. 5—12 illustrate pyriform types with blunt angles or

pointed processes projecting from the sides of the otherwise pyriform parasites.

All these types are peculiar in that they seldom show any movement within the containing corpuscles<sup>1</sup>, and with the exception of the types with the curved tails (2 and 3), even when freed by the rupture of the corpuscle remain stationary. The latter move about in a sluggish manner by means of slow movements of their tails, but never seem to attack corpuscles and soon become motionless. When free in the plasma all these forms remain in the same condition and do not disintegrate for a considerable time.

We have never seen any of these forms enter fresh corpuscles, although we have made repeated observations upon them spending many hours in the study of these forms alone.

Occasionally the pear-shaped processes, which ultimately develop into typical pyriform parasites, exhibit for a time spike-shaped lateral processes as seen in Diagr. 24, which are finally withdrawn. Some of the parasites shown in Diagr. 32 may therefore represent organisms whose development has been arrested.

(7) *The escape of the parasites from infected red blood corpuscles.* In our later experiments we have confirmed and extended our previous observations (p. 622) on the escape of parasites from infected corpuscles. Parasites have now been seen in the act of leaving the corpuscles on many occasions, and it has been noted that three different methods may be adopted. (a) Most commonly the parasite or parasites leave the corpuscle, and the latter immediately becomes pale and finally disappears. (b) Less commonly the corpuscle first becomes pale and then the parasite escapes. (c) On very rare occasions the parasite appears to leave the corpuscle without apparently injuring it.

(a) The mode of escape about to be described in detail is undoubtedly the most common one under experimental conditions, and satisfactorily accounts for the watery condition of the blood and the haemoglobinuria during the last hours of life.

An intra-corpuscular parasite when properly focussed appears as a well defined light body surrounded by a narrow darker zone within the dark corpuscle (see p. 233). No intervening light halo is seen. At times, prior to the escape of the parasite, without any apparent disturbance of the surface of the corpuscle, the parasite seems to disappear as into a fog. Though its general form can still be defined,

<sup>1</sup> We have on many occasions kept such intra-corpuscular forms under observation for hours.

its outline is no longer sharp, and the colour of the organism approaches more nearly to that of the corpuscle. This appearance may be due to the corpuscle assuming a more spherical form owing to the absorption of fluid. Gradually, or at other times rapidly, the parasites become more distinct, show active movements and simultaneously pass out of the corpuscles, often without apparently encountering any great resistance, and swim away. The corpuscle then rapidly loses its colour and almost disappears, although its margin can be still defined by careful focussing. Occasionally, however, no apparent trace of it remains.

Slight differences are noticed in various cases, for example, the foggy stage may not be observed, or the organisms may distort the corpuscle to some extent before their escape as if the envelope offered considerable resistance. At other times the process is so rapid that the various changes can scarcely be followed, and gives the appearance of the parasites being hurled out by the explosion of the corpuscle.

(b) On many occasions the corpuscles were noticed to become pale, or almost invisible before the escape of the parasites. Under these conditions the parasites frequently perform remarkable gyratory movements before leaving the remains of the corpuscle, two instances of which we previously described (x. 1906, p. 623).

While in some cases the parasite seems to encounter very little resistance in passing through the remains of the corpuscular envelope, in other cases it meets with sufficient resistance to alter its shape. A parasite has, for example, been noticed to behave like a blood corpuscle passing through a narrow capillary. A portion of the blunt end apparently passes through an aperture in the corpuscular envelope and the organism gradually forces its way through, without increasing the size of the opening. Consequently that portion of the parasite which is encircled by the walls of the opening is constricted and the whole organism has an hour-glass shape (see *Diagr.* 35. 15).

In connection with these two modes of escape two other appearances of some interest have been noted.

As the corpuscle is fading the surrounding plasma often becomes coloured for an instant on one side of the corpuscle, as if the fluid contents tinged with haemoglobin were being expelled from an opening at the side of the corpuscular envelope. In certain stained preparations the same condition was noticed, confirming the observations made on fresh blood (see *Plate III*).

It was also frequently noticed that after the fading of the corpuscle



certain very small colourless bodies were seen besides the parasites. These sometimes remained within the contour of the faded corpuscles, were sometimes thrown out into the surrounding plasma, or more rarely appeared to be connected to the parasites.

Up to the present we have been unable to decide on the true nature of these bodies. We were at first inclined to believe that they were residual bodies thrown off from the parasites in the process of division. The fact, however, that they have not been recognised in stained preparations is against this view. On the other hand, it is possible that they represent the remains of the stroma of the corpuscle broken up by the movements of the parasites. These minute bodies, whatever their nature, were found to be present in a considerable number of the cases observed, but in other cases, though looked for with the greatest care, not a trace of them could be seen.

(c) In our last paper we described two instances in which parasites apparently escaped from affected corpuscles without causing their destruction (p. 623), but at the same time we pointed out that both these instances might have been accounted for by errors of observation (p. 625). Only two other examples of this mode of escape have been noticed. In one case two pyriform parasites, evidently produced in the usual manner, escaped from opposite sides of the corpuscle which was distorted but soon regained its rounded form, and did not fade. Its border however showed slight undulations. In the other case four pyriform parasites escaped without altering the appearance of the corpuscle.

(8) *The fate of immature parasites.* Our observations have led us to regard the pyriform type as the mature form of the parasite within the blood. These pyriform parasites after the rupture of the containing corpuscles generally attempt to enter other corpuscles in the manner already described (p. 235). If they do not succeed, after a variable period of activity (3—60 minutes or more), they become motionless in the plasma, and in most cases gradually disintegrate. Sometimes these parasites, after slowly fading, suddenly seem to rupture, as it has been occasionally noticed that in the last stages pale, almost invisible, granular material is protruded from them. Some, however, especially the irregular forms described on p. 243, maintain their normal appearance.

When a corpuscle containing one or more (immature) amoeboid or round parasites ruptures the latter almost immediately become round or quiescent, gradually grow indistinct, and finally disintegrate.



After very careful and prolonged observations on this point we are convinced that all parasites thrown into the plasma by the rupture of the containing corpuscle, which have not reached the pyriform stage, sooner or later disintegrate and die. This is even true of parasites which have almost reached maturity, such as those represented in Diagr. 24. 36. In such cases it might be expected that the few remaining changes would be passed through in the plasma, but this is not the case. Even four fully mature parasites attached together by fine threads do not seem to be able to divide, though the process is readily completed within the corpuscle (Diagr. 27. 18—42).

Our further studies tend to confirm the opinion which we expressed in our last paper (1906, p. 601), that the rounded and irregular free parasites found in organ smears are degenerating forms. Many of the larger types (those illustrated, 1906, Diagr. 10) represent parasites in the early stages of division, which have been liberated by the rupture of the corpuscle containing them, and which, as evidenced by the staining properties of their nuclei, are undergoing degenerative changes.

The extent of the destruction or degeneration of the parasites which takes place in the organs may be seen by reference to a previous paper (Graham-Smith, *This Journal*, v, p. 250. 1905), in which the relative proportions of free and intra-corpuscular parasites in the various organs are given.

In the lungs the degeneration is greatest. Smears show a proportion of 1·5 free parasites to each infected corpuscle. In other organs the degeneration is less. Brain smears show one free parasite to each infected corpuscle, smears of lymphatic glands 1—2·2, kidney smears 1—2·2, liver smears 1—2·5, supra-renal smears 1—4·8, marrow smears 1—8, pancreas smears 1—9, and spleen smears 1—9·5.

A small proportion of the free parasites were no doubt fixed at the time they were passing from a ruptured corpuscle to a normal one. In spite of this fact, however, the figures probably represent fairly accurately the proportion of parasites undergoing degeneration in these organs.

In the peripheral blood the number of free parasites is never so great. "Two and more days before death one free parasite to every 38 infected corpuscles were found. The day before death the proportion was one free parasite to 23 infected corpuscles, and on the day of death one free parasite to 18 infected corpuscles" (p. 253).

*Diagram 33* illustrates a prolonged observation on the fate of three intra-corpuscular parasites.

When first noticed 4 minutes after the preparation of the specimen two mature pyriform parasites and one large amoeboid form were noticed within a single red blood corpuscle. During the next  $9\frac{1}{2}$  minutes the pyriform parasites remained motionless, but the amoeboid form threw out pear-shaped processes and appeared to be going to divide into four pyriform parasites. Half a minute later, however, the corpuscle suddenly faded and the contained parasites and two small rounded bodies (see p. 245) were expelled into the plasma (14). Within 11 minutes, during which it exhibited a few movements, the amoeboid parasite became rounded (25), and it remained in this condition until the end of the observation, nearly 2 hours after its escape. On the other hand, both the pyriform parasites (A and B) swam actively away and entered normal corpuscles, A within 3 minutes and B within 2 minutes. Both soon assumed a rounded shape, and afterwards showed slight amoeboid movements. Finally they both again became rounded and motionless. Under natural conditions we have no doubt these parasites would have multiplied in the usual manner.

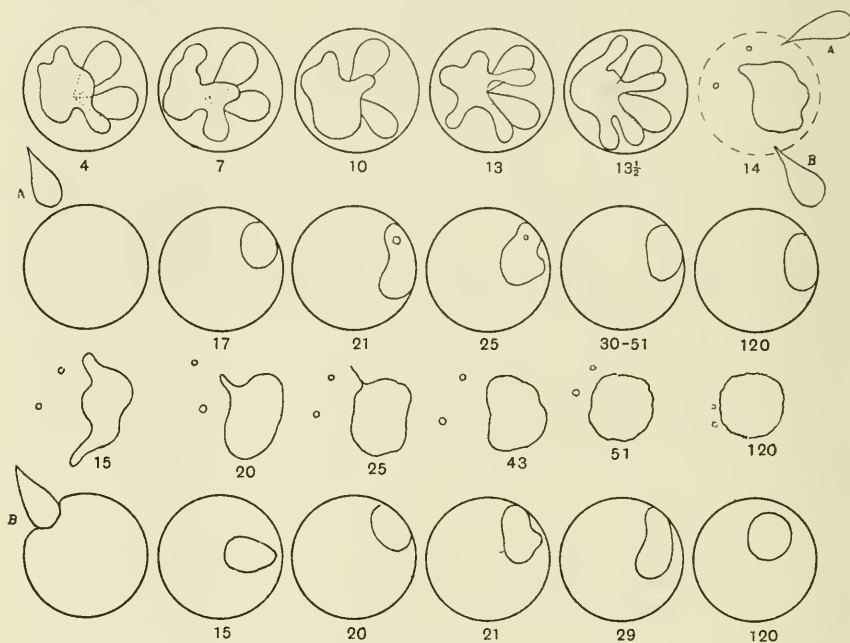


Diagram 33.

(9) *Vacuoles*. In stained preparations clear, well defined, pale areas resembling vacuoles can be seen in a certain number of all

forms of the parasite, but are less common in the actively amoeboid type with pseudopodia. In living preparations vacuoles are also very commonly seen and appear as more or less well defined darker areas of various shapes and sizes within the pale parasites. Plate I, Fig. 1, 11—37, illustrate the changes in the shape of an amoeboid parasite and in the appearance and size of its vacuole during a period of observation lasting 37 minutes.

In the first stage represented, which was drawn 11 minutes after the preparation of the specimen, the vacuole appeared as a well defined large oval area, slightly darker than the parasite. Within half a minute it had contracted to a much smaller size, and its edges had become still better defined. Under these conditions it was a much more noticeable object than before. Shortly afterwards it had moved from the centre to the edge of the parasite, and produced the appearance of an oval notch cut out of the side of the parasite. Subsequently it again moved to the centre of the parasite, and became large and small, and well and ill defined alternately. These changes can be more readily followed by reference to the figures than described.

Vacuoles are indicated in several other figures (Diagrs. 27 and 31) representing living forms, whilst in some they have been intentionally omitted for the sake of clearness.

It is particularly interesting to notice that very large vacuoles are sometimes present in small rounded forms, and that vacuoles of varying size are often present in the symmetrical pear-shaped processes which ultimately develop into mature pyriform parasites. In the latter case the vacuoles still persist in the mature pyriform parasites and are often seen in stained preparations dividing the dense from the loose masses of chromatin.

We have on several occasions made very careful observations on suitable living specimens with Zeiss 2 mm. objectives and high eye-pieces with the purpose of ascertaining whether any evidence of nuclear changes can be obtained. Occasionally darker areas were seen within the protoplasm of some of the parasites, but the significance of such appearances is very doubtful, since with the most careful focussing and adjustment of the light no structure can be made out in many recently liberated pyriform parasites, which, we know from our studies on stained preparations, invariably contain at least one large dense mass of chromatin. Up to the present we have been unable to ascertain anything in regard to the nuclear changes by the study of the living organisms, and the minuteness of the parasites appears at present to be an insurmountable obstacle in the way of solving the problem by this means.

*Summary of observations on living blood.*

*Piroplasma canis* has a free and an intra-corpuscular stage in the blood of the dog, and it is during the latter stage that multiplication occurs. This asexual multiplication takes place in one of the following ways :

(1) A free pyriform parasite which has just left a blood corpuscle enters a normal corpuscle and assumes a round form, remaining quiescent for a time. The round body then grows and, after passing through an actively amoeboid stage, again becomes rounded. Two symmetrical processes are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes assumes a pyriform shape and ultimately gives rise to a mature pyriform parasite, which remains for a time joined to its fellow by a thin strand of protoplasm. On the rupture of the containing corpuscle these pyriform parasites become free and enter other corpuscles.

(2) Occasionally by the protrusion of four pyriform processes four mature pyriform parasites are formed from a single amoeboid form.

(3) Sometimes a young rounded intra-corpuscular parasite divides by simple division and gives rise to two amoeboid parasites, which grow and divide by the protrusion of symmetrical processes each into two pyriform parasites, thus giving rise to four mature pyriform bodies within the corpuscle. Sometimes the two amoeboid parasites undergo the processes of division simultaneously, but not infrequently one is considerably in advance of the other.

(4) It is possible that occasionally a red blood corpuscle is invaded either simultaneously or at different times by two pyriform parasites, each of which undergoes the changes described above.

(5) Several pyriform bodies within a single corpuscle are produced by the division in the manner described of one or more amoeboid parasites.

All parasites which have not reached the mature pyriform stage, when the containing corpuscle ruptures, rapidly degenerate and die in the plasma. The same is true of mature pyriform parasites, which do not, after becoming free, soon enter other corpuscles.

In observations made on living preparations many of the intra-corpuscular parasites, after a longer or shorter period of activity, come to rest as rounded forms near the edges of the corpuscles. This appears to be due to the unfavourable conditions and probably does not occur in the living body.



In an earlier paper (Graham-Smith, 1905, p. 265), a table is given showing the relative frequency of the occurrence of infected red blood corpuscles containing various numbers of parasites, compiled from a number of observations made on organ smears and blood films. Excluding corpuscles containing only single parasites 27,088 infected corpuscles were counted, and of these 26,305 (96·38 %) contained even numbers of parasites (22,286 with two parasites, 3397 with four parasites), and 783 (2·89 %) odd numbers. The mode of multiplication accounts for this disparity.

### (C) The nuclear changes accompanying division.

Aided by the knowledge we had gained of the significance of the various forms assumed during division by the living parasites, we made very careful and extended studies on stained preparations.

In preparing these specimens thin smears were made on thoroughly clean glass slides. Some were fixed (1) by drying in the air, (2) others were immediately plunged into boiling absolute alcohol and a few were fixed either by (3) immersion in a mixture of 1 c.c. of strong formalin to 10 c.c. of alcohol, or (4) by being placed first in formalin vapour for 5 seconds and then immersed in alcohol.

By the first two methods extremely good preparations were obtained, but the other two did not yield as satisfactory results.

All preparations were stained by Giemsa's stain, but the dilution and the period of staining was varied according to the result which was desired.

In some cases dehaemaglobinised films were used, as the finer chromatin strands are more easily studied when the pink tint imparted to the corpuscles by the presence of the haemoglobin is removed.

In our last paper (1906, p. 590) we pointed out that in many parasites several distinct masses of chromatin can be recognised, a large, dense, compact mass, and a lightly staining, irregular, loosely packed or reticulated mass or masses, which had not been previously described. These masses have been regularly noticed in our later observations, and we have been able to determine their relations to each other.

By most observers the dense mass has been described as the nucleus, but owing to the peculiar manner in which the chromatin division takes place, we prefer at present not to give it any definite name.

Schaudinn (1904, p. 438) briefly called attention to the presence of a



third small compact punctiform chromatin body, which he called the blepharoplast. Lühe (1906, p. 47) confirmed this observation, and in our last paper (1906) we gave several figures illustrating it.

In our later observations, however, we have seldom encountered this body, and do not consider that it is a structure of any significance in the division of the parasites. In many cases it seems to represent the loose chromatin in a condensed form.

At first our studies were confined to the final stages of the development of two pyriform parasites from the period when the two symmetrical processes are first protruded to the completion of two mature parasites as shown on pp. 237—238 in Diagr. 24 (30—83) and 25 (21—45). All these stages can be readily identified in stained preparations, and the nuclear changes, peculiar to each, easily studied. We are, therefore, in a position to state without hesitation that the division of the chromatin takes place in the manner described below.

When the actively amoeboid stage is past and the parasite assumes a round form just before the protrusion of the symmetrical processes (Diagr. 25. 21), there projects from the large dense central mass of chromatin a thin strand of chromatin which bifurcates near its extremity. Each branch ends in a small knob of dense chromatin (Diagr. 34. 7, Plate II, Figs. 6, 33). In specimens which have developed further and show the earliest indications of the symmetrical processes it is found that a terminal branch of the chromatin thread passes into each (Diagr. 34. 8, Plate II, Figs. 7, 18, 34). In specimens at a later stage, showing larger processes, the same condition is noticed (see p. 258, Diagr. 34. 9, Plate II, Figs. 7, 8, 18, 19). At this stage a strand of chromatin passes out of the main mass in the body of the parasite and bifurcates at the base of the processes. From the point of bifurcation a strand passes down into each process and ends, usually near its extremity, in a small knob.

By the time the parasite has reached the trilobed stage (Diagr. 24. 33) the main mass of chromatin has altered its position, and (drawn down as it were to the bifurcation by the contraction of the connecting strand) is now situated at the base of the processes. The strand, which connected the main nuclear mass to the branches passing into each process, consequently disappears at this stage (Diagr. 34. 10, Plate II, Figs. 9, 10, 11, 18).

By the time the parasite has reached the stage of division corresponding to that shown in Diagr. 24. 36, the main mass of chromatin has almost divided and is represented by two incompletely separated

masses lying side by side, from each of which a strand passes into a pear-shaped process (Diagr. 34. 11, Plate II, Figs. 12, 35).

A little later the two incompletely separated masses just mentioned move apart, but remain connected by a thin strand (Diagr. 34. 12, Plate II, Figs. 13, 36).

By the time the parasite has reached the condition shown in Diagr. 24. 40, the connecting strand between the two large masses of chromatin has disappeared and each of these has definitely moved into the neck of the pyriform process (Diagr. 34. 13, Plate II, Fig. 20). During these last stages (Diagr. 34. 12, 13) another change has been taking place. The originally thin strand of chromatin, which extended down each process, gradually loses its sharp outline and usually becomes broken up, especially at its knob-like extremity, and now has a loose appearance. At this stage, therefore, the chromatin in the now almost completely formed pyriform parasite has a comet-like appearance, the head of the comet near the apex of the parasite being represented by the dense mass of chromatin and the tail by the loose mass (1906, Plate XI, Fig. 31; Plate II, Figs. 13, 15).

During the further stages in the formation of the two pyriform parasites, and even after their liberation from the corpuscle, as free parasites, the chromatin remains in the same condition (Diagr. 34. 13, 14, 15, Plate II, Fig. 46).

Sometimes that portion of the chromatin strand which projects from the main mass retains its thread-like appearance and only its distal extremity becomes loose in structure (Diagr. 35. 13, 14, above; 1906, Plate XI, Fig. 23; Plate II, Figs. 39, 40). At other times this portion almost completely disappears and there is scarcely any connection to be seen between the dense and loose masses (1906, Plate XI, Figs. 25, 28; Plate II, Figs. 40, 50). More rarely that portion of the chromatin which is usually loose and net-like in structure is represented by a number of small dense granules (Plate II, Fig. 47). Up to the present we have no reason to consider that the variations in the final disposition of the chromatin have any special significance.

When a vacuole or vacuoles are present in the rounded parasite the resulting pyriform parasites are usually also vacuolated. Under these conditions the processes which give rise to these pyriform parasites contain one or more vacuoles at all stages with the possible exception of the very earliest. It is interesting to note that the chromatin almost always has a very definite relation to the vacuoles. In the stages represented in Diagr. 34, Figs. 7 and 8, and Plate II, Figs. 6 and 7, the chromatin

strand runs close to the margin of the vacuoles. During the development of the processes the chromatin strand in each is also closely connected with the vacuoles. When one vacuole is present the strand usually runs round at least one-third and often nearly three-quarters of its circumference (Diagr. 34. 10, Plate II, Figs. 9, 10, 20, 35), and not infrequently, when the strand ends some distance beyond the vacuole, it has a flange-like process passing off at an angle and helping to surround the vacuole (Plate II, Figs. 9, 11, 20). When two vacuoles are present the chromatin strand usually runs between them, often sending off the flange-like processes just mentioned to partially surround one or both vacuoles (Plate II, Figs. 12, 13, 36).

When a vacuole persists in the mature pyriform parasite it either lies at one side of the loose chromatin mass (Diagr. 34. 14, Plate II, Fig. 40) with the tail of the latter curving round it, or separates the dense from the loose mass (1906, Plate XI, Fig. 29; and Diagr. 1. Fig. 4), or most rarely is almost completely surrounded by the chromatin (Plate II, Fig. 37).

Consequently, in regard to the disposition of the secondary masses of chromatin, at least six well-marked varieties of mature pyriform parasites are found, (1) those showing a large loose mass of chromatin connected to the main rounded dense mass near the pointed extremity, (Plate II, Fig. 15; 1906, Plate XI, Figs. 30, 31, 18, and Diagr. 1. Fig. 3; Diagr. 3. Fig. 2; Diagr. 6. Figs. 8, 9, 10); (2) those showing the loose mass near the blunt extremity connected to the main mass by a thin strand of well defined chromatin (Plate II, Fig. 39; 1906, Plate XI, Fig. 23, and Diagr. 3. Fig. 12 and Diagr. 6. Fig. 10); (3) those in which the dense and loose masses are separated from each other (Plate II, Fig. 40; 1906, Plate XI, Fig. 20, 25; Diagr. 1. Figs. 1, 2, 5, 14; Diagr. 3. Figs. 3, 4, 6, 7); (4) those in which that portion of the chromatin which is usually loose in structure is represented by dense granules (Plate II, Fig. 47; 1906, Plate XI, Fig. 24; Diagr. 3. Figs. 8, 10, 22); (5) those in which a distinct vacuole is more or less interposed between the dense and loose chromatin (1906, Plate XI, Fig. 29, and Diagr. 1. Fig. 4); and (6) those in which the loose chromatin is more dense in structure than usual, and is closely applied to the dense mass (rare forms) (Plate II, Fig. 48; 1906, Diagr. 3. Fig. 1).

The secondary masses of chromatin are usually arranged in a similar manner in the two mature pyriform parasites derived from a single amoeboid parasite, but this is not always the case.

Although the various arrangements of the secondary masses of

chromatin in the mature pyriform parasites probably do not represent differences in function, and may perhaps be accounted for by the movements of the protoplasm during their formation, they are of importance when considering the distribution of the chromatin in the small rounded forms to which the pyriforms give rise after entering fresh corpuscles.

The nuclear changes we have hitherto described can be followed without difficulty owing to the characteristic appearances of the parasites during these phases of division, but those changes which occur between the entry of the parasite into a normal corpuscle and the end of the actively amoeboid stage cannot be followed so easily. This is owing to the difficulty of deciding what stage a small rounded intra-corpuscular parasite has reached when seen in a stained preparation.

We confidently believe, however, that in the following pages these changes are correctly described.

Shortly after its entry into a normal corpuscle the pyriform parasite assumes a rounded shape. At this stage the chromatin is arranged in exactly the same manner as it was in the free pyriform parasite (Diagr. 34. 2). Within a short time, while the parasite is still small, the loose chromatin moves towards the main mass (Plate II, Fig. 1), and finally the whole becomes aggregated into one large dense mass (Plate II, Fig. 2). The parasite in some cases has become actively amoeboid before the process is completed. The varying periods at which complete fusion occurs is well seen by reference to a diagram in our last paper (1906, Diagr. 9) illustrating several amoeboid parasites, some with single masses of chromatin (Figs. 3, 7, 11, 12, 13, 15, 18), and others with both loose and dense masses (Figs. 6, 14, 17). During this process different appearances will be seen according to the differences in the arrangement in the pyriform parasites which entered the corpuscles.

Diagr. 34. Figs. 2, 3, 4, 5 (Plate II, Fig. 23; 1906, Diagr. 2. Fig. 4) schematically represent these changes when the entering parasite possesses a vacuole. Diagr. 35. Figs. 1—5 (upper row, Plate II, Figs. 22, 25, 26) represent the changes when the main mass of chromatin of the entering parasite is attached to the loose mass by a thin strand of chromatin, and the lower row of figures the changes when the loose and dense masses are connected without the interposition of a strand.

Subsequently, in most cases towards the end of the actively



amoeboid stage, the large mass of dense chromatin, formed by the fusion of all the chromatin substance, divides into two, often unequal, masses, which remain joined by a thin strand (Diagr. 34. 6; Plate II, Figs. 3, 4, 5; and 1906, Diagr. 2. Figs. 2 and 16). Later the smaller of these two masses again divides. By this means the peculiar Y-shaped chromatin figure already described is formed, which is found in a parasite about to protrude the two symmetrical processes which ultimately give rise to the new pyriform parasites (Diagr. 34. 7, Plate II, Fig. 6).

From this point to the completion of division the nuclear changes have already been described (p. 252).

When the young parasite divides by simple division, and gives rise to two small amoeboid parasites, the process is preceded by the complete division of the chromatin mass (Diagr. 36. 4; Plate II, Figs. 16, 17; 1906, Diagr. 2. Fig. 13).

Subsequently each separate parasite behaves exactly like a single dividing form (Diagr. 36. 6—10; Plate II, Figs. 18, 19, 20).

The arrangement of the chromatin in the irregular forms of intra-corpuscular pyriform parasites deserves a brief description.

The very large pyriform type shown in Plate II, Fig. 42, usually possesses very little chromatin which is situated in small widely separated masses (see also 1905, Plate 9, Fig. 13).

The form showing a spike-like process (Plate II, Fig. 43) generally exhibits similar masses of chromatin to those seen in mature pyriform parasites.

The large irregular parasites represented in Plate II, Fig. 44, each possess a single mass of dense chromatin and an extensive network of loose chromatin. These forms are of particular interest, because in one instance similar shaped organisms were seen to give rise to several pyriform parasites (p. 240).

When suitable opportunities occur for the observation of pyriform parasites lying on their edges, it can frequently be seen that the dense mass of chromatin causes a distinct prominence on the surface of the organism. Such a condition is illustrated in Plate II, Fig. 46.

It has generally been asserted that polychromatophile degeneration is not found in canine piroplasmosis, and the condition is undoubtedly very rare. In one of our cases, however, a large number of corpuscles were affected, one of which is represented in Plate II, Fig. 48.



**(D) The complete cycle of development within the blood.**

In a previous paper (1906), and on page 235 of the present paper, we have fully described, with the aid of drawings made during the observations, the multiplication processes which we have studied in the living blood of dogs suffering from acute canine piroplasmiasis. Since, in the course of very prolonged and careful investigations, we have never seen indications of any other methods of reproduction than those we have described, we believe that, whatever the number of parasites produced within single red blood corpuscles, the process in all cases is essentially similar. Further, we believe that multiplication never occurs outside the corpuscles, and that all immature parasites, which are liberated by the rupture of the corpuscles which contains them, and all mature pyriform parasites, which fail to enter fresh corpuscles degenerate and die.

The nuclear changes which occur in the parasites during the process of growth and division within the red blood corpuscles, and the various arrangements of the secondary masses of chromatin in the mature forms, have been described in the last section.

The results of all our observations on living and stained specimens are summarised in the present section by the aid of schematic figures which indicate the principal changes in form which the parasites undergo in the processes of multiplication and passage from one corpuscle to another and the nuclear changes which accompany them.

*Description of Diagrams 34—36.*

*Diagram 34.* Fig. 1 (p. 258), shows a free pyriform parasite about to enter a normal red blood corpuscle which it is indenting (1906, Plate XI, Figs. 17, 18, etc.). The parasite contains a vacuole, and possesses a single dense mass of chromatin connected with a loose mass near its blunt end by a thin strand.

In Fig. 2 the parasite is represented as having entered the corpuscle and become rounded in shape, while the chromatin still retains its original disposition (Plate II, Fig. 23).

In Fig. 3 the parasite has grown and its vacuole has enlarged, but the chromatin has not undergone any alteration.

In Fig. 4 the parasite has still further enlarged, and the loose mass of chromatin has been drawn up to, and become condensed close to the dense mass (Plate II, Fig. 1).

In Fig. 5 the whole of the chromatin has fused, and a secondary loose mass can no longer be differentiated (Plate II, Fig. 2).

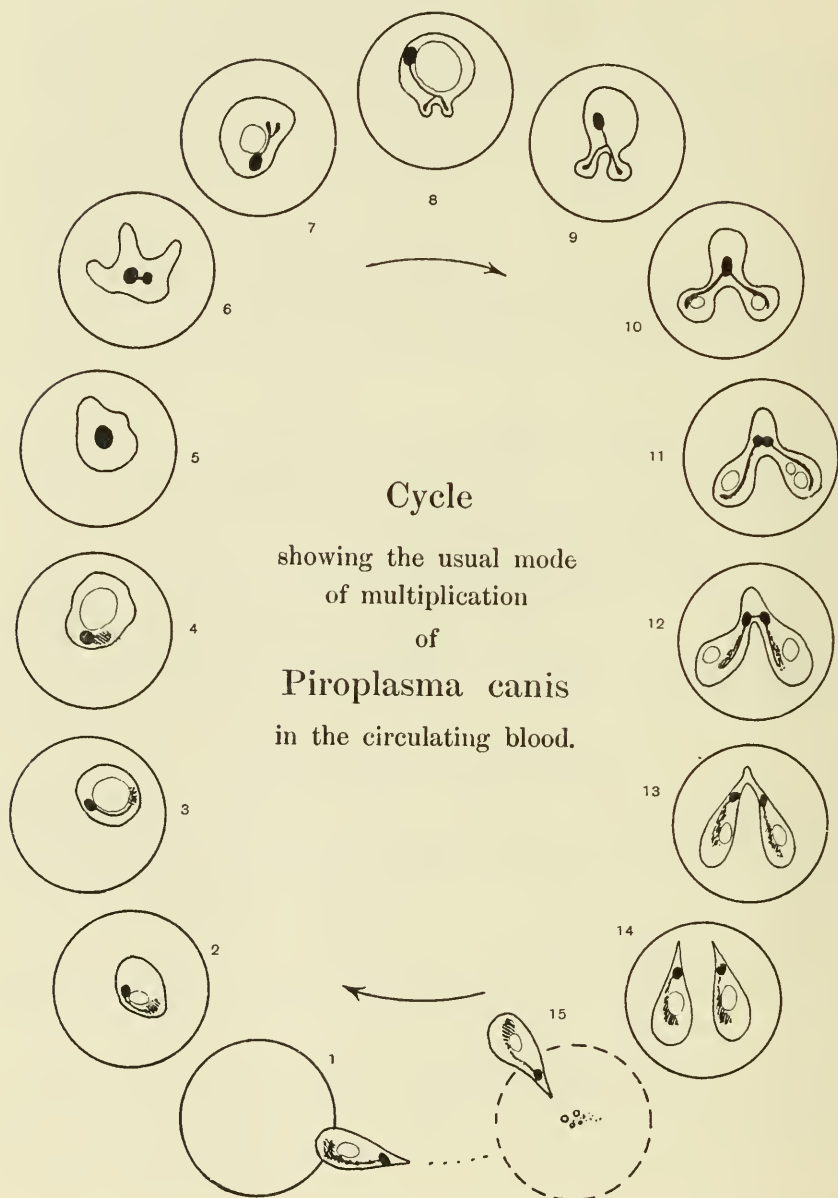


Diagram 34.

In Fig. 6 the parasite is represented in the amoeboid stage with three pseudopodia. The single chromatin mass has by this time divided into two unequal sized masses, connected together by a thin strand (Plate II, Figs. 3, 4, 5; 1906, Plate XI, Fig. 8). For the sake of simplicity the long amoeboid stage is represented by one figure only, and for the same reason the vacuole has been omitted in this and the previous figure. During the earlier stages represented in Figs. 1—4 and the later stages shown in Figs. 7—15, the vacuole, when present, is closely related to the chromatin, which almost invariably lies along its margin. In those stages, however, in which the whole of the chromatin is gathered together into a central mass, no special relation to the vacuole has been noticed.

In Fig. 7 the parasite is represented in the rounded quiescent stage after the cessation of the active amoeboid movements. At this stage the two masses of chromatin shown in the last figure have moved apart and in the smaller has again divided. The three main masses thus produced are still connected together by a thin strand, which runs from the main mass close along the edge of the vacuole and eventually bifurcates to send a branch to each of the divisions of the smaller mass (Plate II, Figs. 6, 33). At this stage the parasite shows no processes.

In Fig. 8 two small symmetrical processes have been protruded by the parasite, each supplied with one of the divisions of the smaller chromatin mass (Plate II, Figs. 7, 18, 19, and 34).

In Fig. 9 the processes have enlarged, but the general arrangement of the chromatin remains the same as before (Plate II, Fig. 8).

In Fig. 10 the trefoil stage (see Diagr. 24. 33) is represented. By this time the main chromatin mass has altered its relation to the body of the parasite and to the rest of the chromatin. It is no longer situated at a distance from the processes, but has moved to a position near their bases. During this movement the chromatin strand, which connected the main mass to the two branches passing into each process, shortens and disappears, so that finally the latter branches project directly from the main mass (Plate II, Figs. 9, 10, 11, 18, 20).

An attempt has also been made to indicate the relation of the chromatin to the vacuoles which appear in the processes about this stage. This relationship has already been described (p. 253), and is better seen by reference to Plate II, Figs. 9, 11, 12, 13, 20, 35, 36.

In Fig. 11 the processes have still further enlarged at the expense

of the body of the parasite, and the main mass of chromatin situated near their bases shows signs of division (Plate II, Figs. 12, 35).

In Fig. 12 the division just mentioned is represented as nearly completed, but the resulting masses are still connected by a thin strand. At this stage a change takes place in the appearance of the strands of chromatin passing down the processes. They lose their definite contour and become transformed into masses of loose chromatin with a reticular structure (Plate II, Figs. 13, 36).

In Fig. 13 the original body of the parasite has almost completely disappeared, and the whole of the chromatin has passed into the processes. The dense masses resulting from the division mentioned in Figs. 11 and 12 become completely separated, and finally take up their positions near the tapered extremities of the processes (Plate II, Fig. 20).

In Fig. 14 the completely formed pyriform parasites, resulting from the division of the single parasite which entered the corpuscle (Fig. 1), are shown. Each possesses a dense mass of chromatin near its pointed extremity, from which a tail of loose chromatin passes towards the blunt extremity. The latter is closely apposed to the margin of the vacuole (Plate II, Figs. 15, 19, 20, 38, 39, 40).

In Fig. 15 the escape of these two parasites is indicated, and one is represented as passing into another corpuscle (Fig. 1). The discontinuous line represents the contour of the ruptured corpuscle. In the centre of the latter some granular matter is shown, which may either represent residual protoplasm cast off by the parasite during division, or the remains of the stroma of the corpuscle (see p. 246).

*Diagram 35* supplements *Diagr. 34*, giving two of the variations in the disposition of the secondary or loose chromatin, which may occur subsequent to the stage represented in the latter in Fig. 12.

Fig. 12 (right-hand central Fig.) is in all respects similar to Fig. 12, *Diagr. 34*, except for the fact that no vacuole is represented. The upper row of figures represents the appearances which are seen when the terminal portions only of the strands of chromatin, which pass down the processes, are changed into reticular masses. In Fig. 14 the completely formed parasites of this type within the corpuscle are represented (Plate II, Figs. 39, 40), and in Fig. 15 the rupture of the corpuscle and the escape of the parasites, one of which is in the hour-glass condition while passing through the corpuscular envelope

(see p. 245). Figs. 1, 2, 3, 4, and 5 show the gradual condensation of the chromatin into a single mass after the entry of one of the parasites into a fresh corpuscle (Plate II, Fig. 23).

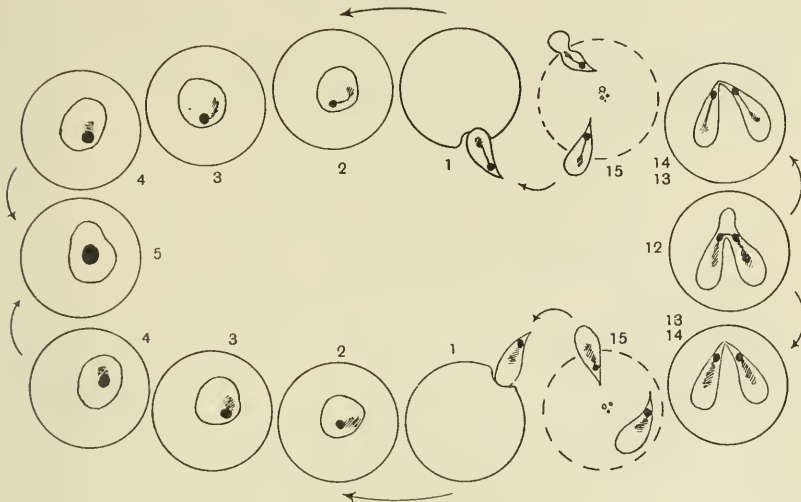


Diagram 35.

In the lower row of figures the same series of events are represented in parasites without vacuoles, in which the secondary mass of chromatin forms a loose mass closely related to the dense mass (Plate II, Figs. 15, 45).

The subsequent changes are similar to those shown in *Diagr. 34*. Figs. 6—15.

*Diagram 36* indicates two methods by which four pyriform parasites may be produced within a single corpuscle.

In *Fig. 1* a single pyriform parasite is shown entering a corpuscle.

In *Fig. 2* the same parasite is represented after its entry with the corpuscle. In *Fig. 3* the chromatin has become condensed into one mass. Up to this point the parasite has behaved in the same manner as that represented in *Diagr. 34*. Figs. 1—5. In *Fig. 4* the parasite is shown in the act of dividing into two small rounded parasites (Plate II, *Fig. 16*), the nucleus having already divided. In *Fig. 5* the resulting two small round parasites, each with a single dense mass of chromatin, are shown (Plate II, *Fig. 17*). In a previous paper (1905, Plate IX, Figs. 3, 4, 5, 6, and 7) the appearances during



these stages have been well represented. Consequently, we have not reproduced similar figures in the plate accompanying this paper. After this each parasite behaves in the same manner as the one shown in Diagr. 34. Fig. 6 corresponds to Diagr. 34. Fig. 5; Fig. 7 to Diagr. 34. Fig. 9; Fig. 8 to Diagr. 34. Fig. 10; and Figs. 9 and 10 to Diagr. 34. Figs. 12, 13, and 14 (Plate II, Figs. 18, 19, 20)<sup>1</sup>. The escape of the four pyriform parasites is shown in Fig. 11 (Plate III).

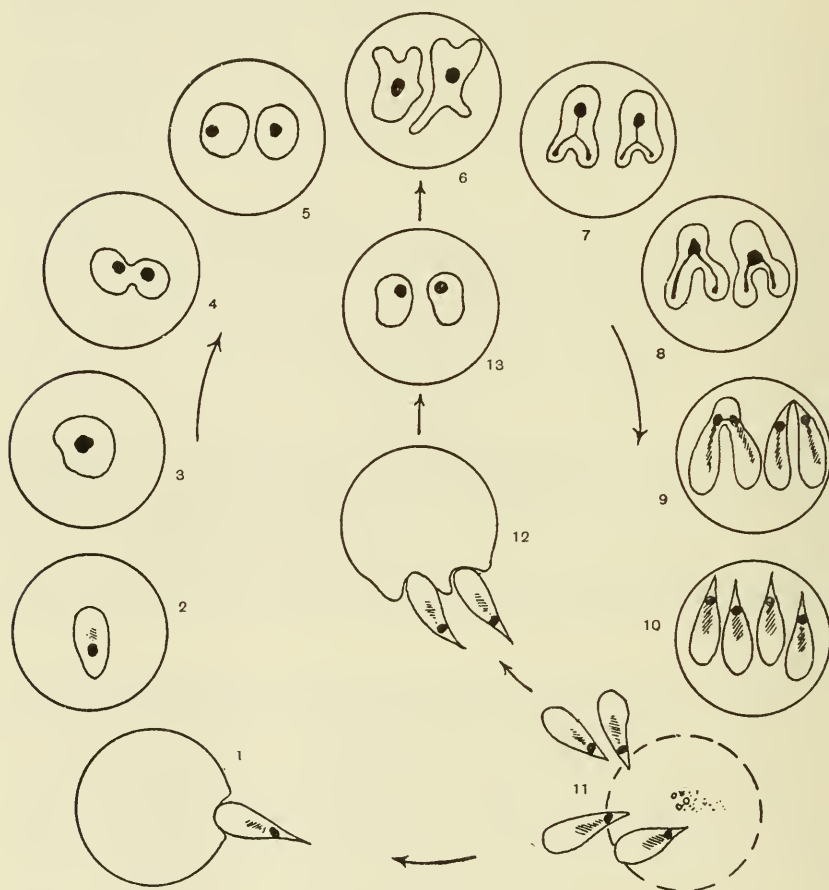


Diagram 36.

<sup>1</sup> Various stages in this process are clearly represented in some of the figures of our last paper (1906). In Diagr. 5. Fig. 6, two small parasites are shown, one with a single mass of chromatin, and one with two masses joined by a strand. In Fig. 6 two parasites are shown each with two masses joined by a strand. In Figs. 9 and 10 later stages are represented.

We believe that this is the usual mode of formation of four parasites within a single corpuscle.

Although we have never observed the entry of two parasites into a single corpuscle, it does not follow that such an event never occurs within the dog's body, when the corpuscles are closely packed together in the slow current of capillaries. In such a case each parasite would probably soon become rounded and retract its chromatin into a single mass (Fig. 13), subsequently these parasites would, we assume, behave in the same manner as two small parasites derived by division from a single parasite (Figs. 6—11).

#### *Conclusions.*

With the exception of a few observations on points which have little or no bearing on the development of the parasites, we have brought to a conclusion our experiments on that part of the life cycle of *Piroplasma canis* which is passed within the blood of the dog. Our observations on the living blood alone, conducted at all stages of the disease and at all hours of the day between 9 a.m. and midnight, have occupied more than 550 hours of careful study.

We therefore think that we are justified in considering that none of the common phases at any rate, which can be followed under experimental conditions, have escaped our notice. As we have already pointed out, our observations lend no support to any of the theories of development which have hitherto been put forward (see 1906, pp. 641—643). Most of those appearances which give rise to erroneous impressions have been briefly recounted in a previous paper (1906, pp. 604—610) and certain others are given in the appendix to this paper (p. 268).

Our observations on the mode of development of single free parasites, which enter red blood corpuscles, into two mature parasites, and on the methods by which several pyriform parasites are produced in single red blood corpuscles are summarised at length in this paper (p. 250).

We, however, briefly recapitulate the former process here :

A free pyriform parasite enters a normal red blood corpuscle and rapidly assumes a rounded form. It then enlarges and passes through an actively amoeboid stage, at the end of which it again becomes rounded. After a short period of quiescence in this condition it protrudes two symmetrical processes, which rapidly grow and become

pear-shaped. The protoplasm of the parasite flows into these processes, and its body consequently gradually diminishes until it is represented by a minute rounded mass to which the pyriform processes are attached. Eventually this also disappears, and finally two mature pyriform parasites are left, which are joined together for a time by a thin strand of protoplasm. After a variable time these parasites are liberated by the rupture of the corpuscle, and swim away to enter fresh corpuscles and repeat the process.

Occasionally a single rounded intra-corpuscular parasite by the protrusion of several processes, such as have just been described, gives rise to four or more mature parasites, or a single parasite divides into two small rounded parasites, each of which produces two pyriform parasites.

Under experimental conditions all parasites, which are liberated by the rupture of the corpuscles containing them before they have reached the mature pyriform stage and all mature pyriform parasites which fail to quickly enter fresh corpuscles, disintegrate and die.

Observations on stained preparations lead us to conclude that from this cause a great destruction of the parasites takes place in the living body, especially in the organs. Under the conditions of observation many parasites fail to become fully developed in the corpuscles, and come to rest as rounded forms, but this probably does not occur as frequently in the living body.

Aided by the knowledge we had gained by the study of living preparations, we have made very prolonged and careful examinations of stained specimens, and have been able to work out the nuclear changes which accompany development. These changes have been summarised at length in section (D) (p. 257).

Briefly these changes are as follows:

The free pyriform parasites possess a mass of dense chromatin near their pointed extremity and a secondary mass of loose chromatin extending towards the blunt end, which may be arranged in various ways. When the parasite becomes rounded within a corpuscle the original arrangement of chromatin is retained for a time. Gradually the two masses become approximated, and either before or during the amoeboid stage become fused into a single dense mass. This mass later divides, but the resulting masses remain united by a thin strand of chromatin. Just before the protrusion of the symmetrical processes, one of the latter masses again divides in such a manner that a peculiar Y-shaped chromatin figure is formed. This consists

of a large dense mass from which a thin strand projects, which bifurcates at some distance from the large mass, sending strands to two small masses. When the processes are formed one of these smaller masses passes into each, still remaining united with the larger mass. At a late stage in the division of the parasites the main mass of chromatin also divides, and a portion passes into each process, ultimately giving rise to the dense mass in the mature pyriform parasite. The smaller mass and the connecting strands give rise to the secondary mass.

When a small round intra-corpuseular parasite divides into two small parasites, the process is preceded by the simple division of the chromatin. In the subsequent development of these parasites the chromatin behaves in the manner described above.

We have never observed any forms which could be regarded as gametes (see 1906, p. 640, footnote).

### DESCRIPTION OF PLATES I—III.

PLATE I. Has been described sufficiently in the text under section (A), pp. 233—234.

PLATE II. The specimens illustrated in this plate were painted with the greatest possible care to depict as accurately as possible every structure which was visible.

When a suitable specimen had been found under the microscope (2 mm. Zeiss apochromatic oil immersion and 6, 8, 12, and 18 compensating oculars with artificial light) it was sketched as accurately as possible by one observer (G.-S.). The specimen was then painted by the other observer (N.) and finally the two figures were compared and the details discussed. Unless both observers were in complete agreement upon the details of every structure the specimen was rejected.

We can, therefore, confidently assert that the figures accurately represent the appearances seen in preparations carefully stained by Giemsa's method.

Figs. 1—15 show the stages in the formation of a pair of pyriform parasites from a single rounded form.

Fig. 1 shows a small intra-corpuseular form in which the loose chromatin has approached the dense mass.

Fig. 2 shows a later stage in which all the chromatin has fused into a single dense mass.

Figs. 3, 4, and 5 show three stages in the division of the single mass into two masses joined by a thin strand, which soon become widely separated.

Fig. 6 shows the parasite after the completion of the active amoeboid stage. One mass of chromatin has again divided giving rise to two small knob-like masses joined by strands to the single strand which projects from the main mass.

Fig. 7 represents an early stage in the formation of the symmetrical processes. One of the knob-like masses seen in the last figure is situated in each process, but still remains connected in the same manner as before to the main mass. While the body of the parasite possesses a large vacuole a small one is contained in each process.



Fig. 8 represents a condition in which the processes have enlarged, but the general disposition of the processes remains the same as before.

Fig. 9 represents a still more advanced condition. Here the main mass of chromatin has taken up its position near the bases of the processes and is directly connected to the strands passing into each.

Figs. 10 and 11 represent further stages in the growth of the processes. The relations of the chromatin remain unaltered.

Fig. 12 represents the earliest stage in the division of the main mass of chromatin. It is now represented by two rounded closely connected masses.

Fig. 13 represents the stage in which the body of the parasite has almost disappeared and the divisions of the main mass of chromatin have moved apart but are still connected by a thin strand. The secondary mass of chromatin is no longer thread-like but is loose and widely distributed.

Fig. 14 represents a slightly more advanced condition in which the chromatin in one process is no longer connected to that in the other.

Fig. 15 represents two mature intra-corpusecular parasites each with a rounded dense mass of chromatin near its apex, and a loose mass extending from it.

Figs. 16—20 represent the stages in the formation of two pairs of pyriform parasites.

Fig. 16 represents a small parasite in the act of dividing.

Fig. 17 shows the completion of such a division resulting in two small rounded parasites within one corpuscle (see also 1905, Plate IX, Figs. 3, 4, 5, 6, and 7, and description, p. 248).

Fig. 18 represents an early stage in the production of four pyriform parasites from two amoeboid parasites. The left-hand specimen is slightly further advanced than the right-hand one.

Fig. 19 represents another example of the same process. The corpuscle contains two mature parasites which have developed from one amoeboid parasite, as well as an amoeboid parasite in an earlier stage of development.

Fig. 20 represents a corpuscle containing two almost mature parasites, a parasite in an advanced stage of development into two parasites, and a rounded form which has not yet completed the condensation of its chromatin.

Fig. 21 represents a rounded intra-corpusecular parasite with a large dense mass of chromatin and a smaller separate mass ("blepharoplast" of Schaudinn and Lühe).

Fig. 22 represents a young intra-corpusecular parasite before the condensation of the chromatin. This was evidently derived from the form of parasite shown in Fig. 39, since it possesses a dense mass of chromatin attached to a loose reticular mass by a thin strand.

Fig. 23 represents a parasite in the same stage as that shown in the preceding figure. Here a vacuole, surrounded by thin strands of chromatin, is interposed between the dense and the loose chromatin. This form is derived from the kind of parasite shown in one of our previous papers (1906, Plate XI, Fig. 29, or Diagr. 1. Fig. 4).

Figs. 24, 25, and 26 probably represent varieties of the type shown in Fig. 22.

Figs. 27 and 28 probably represent stages in the condensation of the chromatin in intra-corpusecular parasites derived from such pyriform parasites as are seen in Figs. 37, 38, 47; 1906, Plate XI, Fig. 24.

Fig. 29 appears to represent a later stage in the development of the form shown in Fig. 23, when the vacuole has enlarged and caused the chromatin surrounding it to become thinned out.

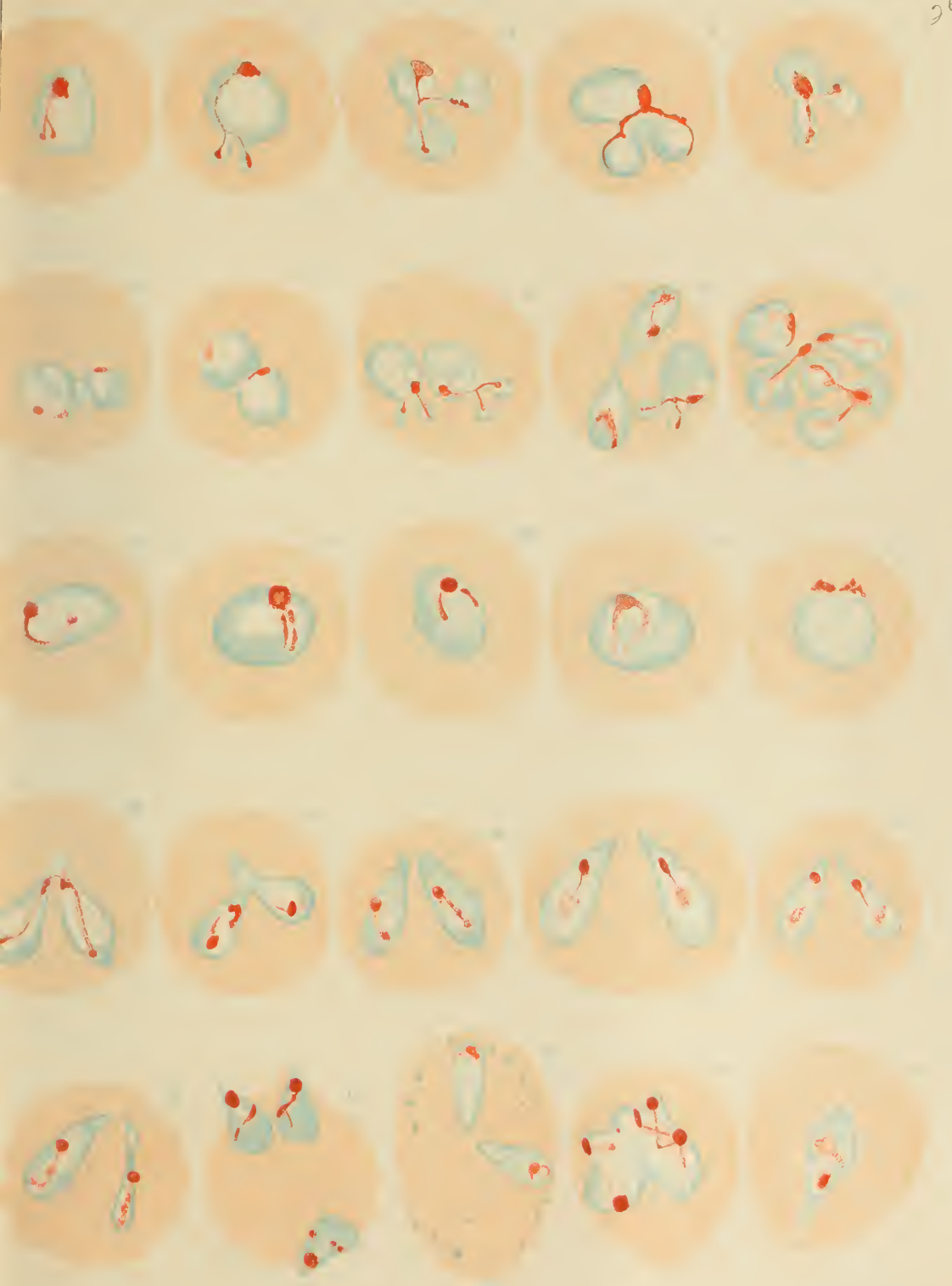
Fig. 30 probably represents an abnormal type of the division seen in Fig. 3.

Fig. 31 probably represents an early stage in an atypical form of the division of the

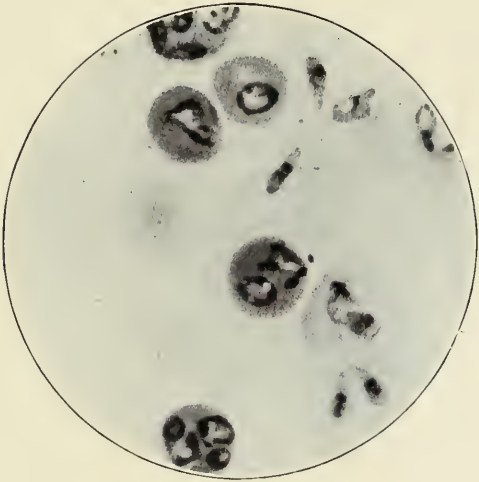
















second mass of chromatin derived from the division shown in Fig. 3 and corresponds to the stage represented in Fig. 6. The division results in one small mass moving away from the other, which remains in connection with the original strand.

Fig. 32 represents a more advanced stage in the same atypical mode of division.

Fig. 33 shows an unusual form of the condition represented in Fig. 6.

Fig. 34 represents a more advanced stage of the process seen in Figs. 31 and 32. One of the processes which has formed, contains a strand of chromatin with a small mass at its extremity, while the mass which should have moved into the other process retains its original position. We think that on further growth the second mass would pass into its process, and develop the usual connecting strand. If this took place a parasite resembling that shown in Fig. 7 would be formed.

Fig. 35 represents a parasite in the same stage as that shown in Fig. 12. It shows extremely well the encircling of the vacuoles in the processes by the chromatin.

Fig. 36 represents a parasite in the same stage as that in Fig. 13 but with a slightly different arrangement of chromatin.

Fig. 37 represents a parasite in the same stage as that shown in Fig. 14. The chromatin is disposed in a very unusual manner.

Figs. 38, 39, and 40 represent mature intra-corpuscular parasites with various arrangements of their secondary masses of chromatin.

Fig. 41 represents a parasite in the same stage as that shown in Fig. 11. The secondary mass of chromatin is in an unusually concentrated condition.

Figs. 42, 43, and 44 show irregular forms of mature intra-corpuscular parasites. Those seen in Fig. 42 are typical in shape, but of very large size and possess very little chromatin.

One parasite in Fig. 43 has a spike-like process projecting from its side. The dense and loose masses of chromatin are very close together. The irregular pyriform parasites shown in Fig. 44 are particularly interesting, because in one instance similar shaped organisms were seen to give rise to several regular pyriform parasites. Each possesses a single mass of dense chromatin and an extensive network of loose chromatin.

Fig. 45 represents a common condition. The corpuscle contains two fully mature parasites and one rounded form in an early stage of development.

Fig. 46 shows two pyriform parasites in a slightly crenated corpuscle. One of the parasites is seen on edge, and shows extremely well the projection caused by the dense mass of chromatin.

Fig. 47 shows three intra-corpuscular parasites in the act of escaping. Two of these have pushed the envelope of the corpuscle before them.

Fig. 48 represents two parasites in a corpuscle which shows polychromatophile degeneration.

Fig. 49 represents a parasite with a complicated arrangement of the chromatin, probably about to give rise to several pyriform parasites.

Fig. 50 represents a free pyriform parasite lying on a normal corpuscle. The organism is surrounded by a white halo (see Fig. *a*, Plate I) and has produced a further distortion of the surface of the corpuscle which is evidenced by two white lines running from the pointed end of the parasite to the margin of the corpuscle.

PLATE III. Photograph of a stained preparation of the blood of a dog taken shortly before death. All the corpuscles are infected. In one instance (see line on the right) the parasites have been fixed in the act of escaping. Two free parasites have already left the corpuscle and two are still within the remains. On the side on which the parasites have escaped the corpuscle has lost its haemoglobin, but on the other side some is still left, and the contour of the corpuscle can be distinctly seen. Another corpuscle (in the

field) has almost completely vanished and the two pyriform parasites which have escaped appear widely separated. We are greatly indebted to Mr Max Poser, of the Firm of Carl Zeiss, London, for this remarkable photograph which he has kindly taken from one of our specimens.

### *Appendix.*

In certain of our earlier observations we were surprised to occasionally observe dumb-bell-shaped bodies, which showed well marked movements, as well as rounded bodies with long flagella-like processes often ending in a collection of minute knobs. Despite the most careful observation we did not at that time succeed in tracing the origin of these bodies, nor could we account for the fact that they were sometimes present in one film made from a drop of blood and not in another film made from the same drop.

In our last paper (1906, p. 627; *Diagr.* 21. Figs. 3, 4, and 5) we figured and described some of these bodies which we saw in specimens prepared during the last few hours of life, and which we regarded as free forms of the parasites. At the same time we were careful to point out that we had not been able to follow their development.

Since that time we have made further studies on this question and have succeeded in demonstrating that these bodies are not parasites, but degeneration products of the red blood corpuscles.

Similar appearances had apparently been noted by Durham<sup>1</sup> in his work on trypanosomes, and, although in the books we have consulted, no references are made to the subject, the changes about to be described are doubtless known to physiologists. Consequently, we do not claim that these observations describe hitherto unknown phenomena. We merely take this opportunity of again calling attention to the changes, of which we were completely ignorant, which occur in blood corpuscles under certain conditions. The appearances to which these changes give rise caused us much trouble, and are probably unknown to many workers on these subjects.

When a fresh specimen of normal human blood is mounted by placing a drop on a clean glass slide, and covering it with a cover-glass and ringing the latter round with vaseline, the corpuscles appear as round, pale discs. If the temperature of the preparation is gradually raised in a thermostat to 50° C., the leucocytes become rounded and motionless, but the red corpuscles do not alter. When the temperature

<sup>1</sup> Mr H. E. Durham informs us that he remembers having seen such bodies, but we have been unable to find any reference to them in any publication.

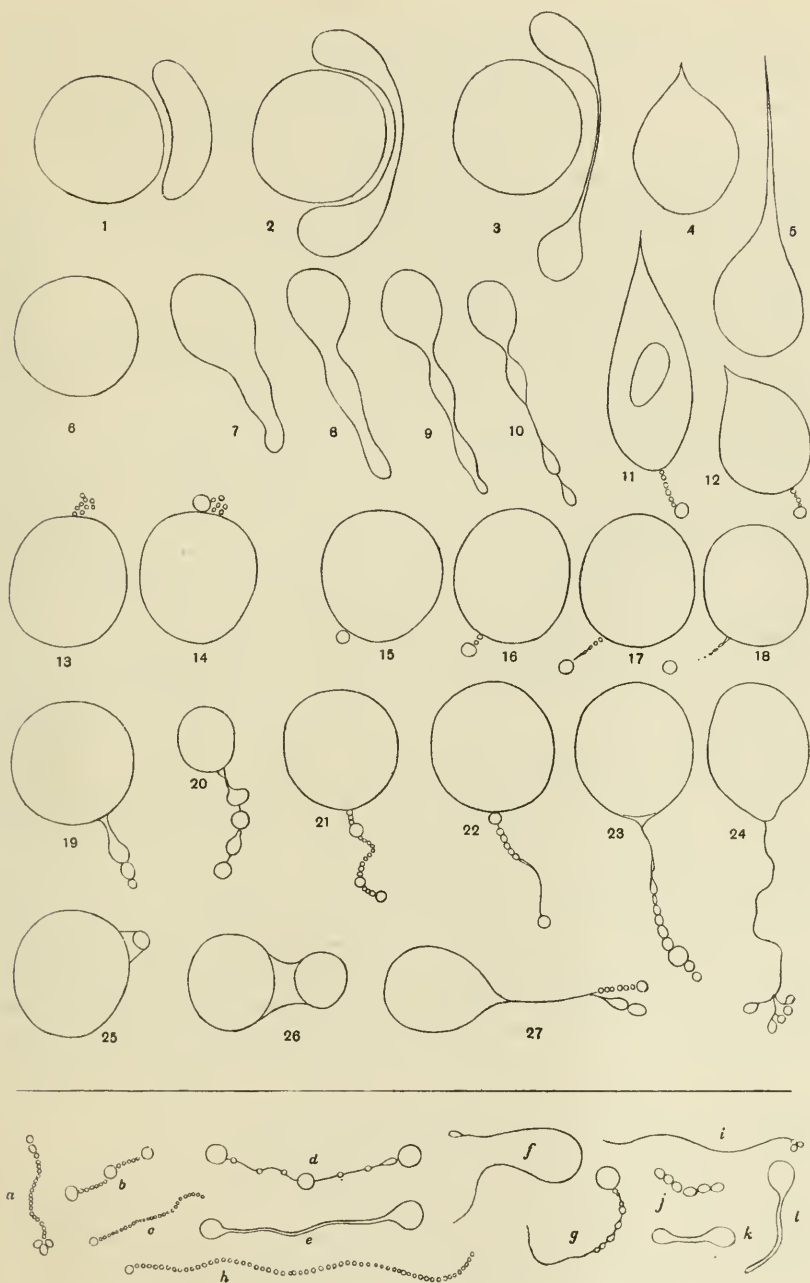


Diagram 37.

is raised to between  $51.5^{\circ}\text{C.}$  and  $52.5^{\circ}\text{C.}$ , however, very marked changes generally occur. Some of the corpuscles suddenly elongate, or show one or more minute round bubble-like protrusions (Diagr. 37. Figs. 4, 5, 6—10, 13, 14, 15). Not infrequently, one of these protrusions is suddenly projected for a considerable distance, but still remains attached to the corpuscle by a thin strand (Figs. 16, 17, 18, 24). Figs. 15, 16, 17, 18 illustrate a case in which a small round mass was first protruded (15), and then rather rapidly moved from the side of the corpuscle while retaining its attachment by means of a strand. At other times the corpuscle elongates and the point seems to stick to the glass and the corpuscle moves away producing the same result (Figs. 6—10). Fig. 6 shows a normal corpuscle which suddenly became elongated (7). The pointed extremity seemed to stick to the glass while the main mass moved away, eventually giving rise to a chain of four unequal sized masses attached to each other by a thin strand (10). Occasionally several small round or elongated masses form the extremity of the projection, and may be united to each other by thin filaments (Figs. 24, 27). These processes are continually in motion, moving from side to side.

Much more commonly the strand is not uniform, but shows swellings of various sizes and shapes along its course. These swellings may be elongated or rounded, large or small, and regularly or irregularly distributed. These various types may alternate in the same strand. Figs. 11, 12, 19, for example, show short irregular protrusions, Fig. 20 a longer example of the same type, Fig. 21 a long strand with many minute and three larger swellings, and Figs. 22 and 23 more irregular types. Sometimes a corpuscle moves from its place and impinges against another which is apparently fixed to the glass. In this case the moving corpuscle may become flattened, and eventually elongated, so as to curve round the other. By this time most of the corpuscular substance is accumulated at the ends, and the central portion is reduced to a thread. In this way a dumb-bell-shaped body is formed (Figs. 1, 2, 3).

Figs. 1—3, 6—10, 15—18 illustrate examples in which the various stages were drawn during the process of formation.

In every case the protrusion of substance causes a more or less considerable diminution in the size of the corpuscle, depending on the quantity of substance extruded (Figs. 6—10). Not infrequently the corpuscles shrink to half their original size, or even less.

Sometimes the corpuscle divides into two rounded masses of equal



or unequal size. These may remain united by very thin, almost invisible, substance (Figs. 25, 26), or become completely separated. They may either remain in this condition, or one or both of them may extrude material in the manner described.

Further, it very frequently happens that the long extended filaments break off either near the original corpuscle or at some distant point, giving rise to free filaments, which show active "wriggling" movements due to the molecular vibrations of the constituent particles.

A few of these are represented in Figs. *a-l*. Some of these bodies, owing to their colourless appearance and active movements closely simulate parasites.

If the specimen is suddenly raised to 52.5° C. the changes just described are extremely well marked. Very few normal corpuscles can be found, and the blood corpuscles are converted into innumerable small round bodies, such as are shown in Figs. 13, 14, mixed with moving filaments, belonging to all the types shown in Figs. *a-l*.

A lesser degree of heat combined with mechanical injury, such as that caused by pressure, brings about similar changes.

Other changes of less interest from our point of view take place at slightly higher temperatures, for example, some of the corpuscles become irregular in shape and then suddenly fade, others split into fragments, and others become crenated.

In the few observations we have made on the blood of different species of animals we have noticed that they differ in regard to the temperature at which this change takes place.

The blood corpuscles of guinea-pigs and rabbits withstand a greater degree of heat than human blood, and do not break up in this manner below 54° C. Normal dog's blood acts in the same manner. We believe that the blood of dogs suffering from piroplasmiasis reacts at a slightly lower temperature. Nucleated blood corpuscles (fowl, frog, Fig. 11) behave in the same way.

The bodies which we have briefly described cannot be easily mistaken, when their origin, movements, and general configuration have once been carefully studied.

In transferring a drop of blood to a slide we made use of a thin platinum loop recently sterilised by heat. In some instances the loop, although apparently cool, must have retained sufficient heat to act on those corpuscles with which it came in contact. Consequently, in the preparations subsequently made, most of the corpuscles appeared normal, but here and there a small degenerated form with a long

filament attached, or a completely detached motile filament (Figs. *a—l*) was seen. Owing to their motility and lack of colour these bodies were at first mistaken for flagellated forms of the parasite. The fact that such forms could not be found in stained preparations, and sometimes only occurred in one out of two films made from the same drop of blood, soon made us realise that they had probably nothing to do with the parasites, but were artificially produced. Nevertheless, we had some difficulty in determining their true nature.

In all our later experiments described in the present paper, this source of error has been carefully excluded.

We hope that this brief note may be of assistance to those who are working on similar subjects.

## ACTION OF THE COLOURS OF BENZIDINE ON MICE INFECTED WITH *TRYPANOSOMA DIMORPHON*.

BY C. M. WENYON, B.Sc., M.B., B.S.,  
*Protozoologist, London School of Tropical Medicine.*

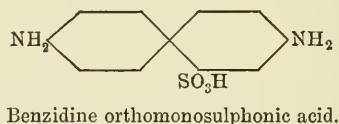
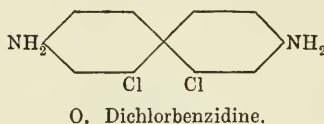
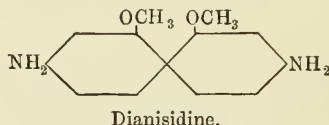
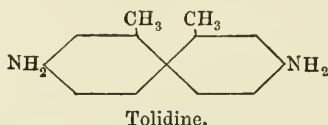
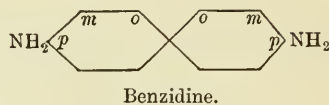
THE investigations which form the subject of this paper were undertaken at the Pasteur Institute, on the suggestion of M. Mesnil. It is a pleasure to acknowledge my great indebtedness to M. Mesnil for the help and advice he so willingly rendered me whilst I worked in his laboratory. M. Nicolle and he very kindly placed at my disposal their unique collection of colours, which had been supplied them by various firms (notably the Farbenfabriken of Elberfeld). By their suggestions, without which this work would not have been accomplished, they saved me much time and labour. MM. Nicolle and Mesnil had been studying the influence of a large number of colours on various trypanosomes, chiefly *Trypanosoma Brucei* and to a less extent *T. Evansi*, *T. equinum*, and *T. Gambiense*. Their work was undertaken after the publication of Ehrlich and Shiga's paper on the action of Trypanroth, one of the benzidine colours, on *Trypanosoma equinum*. M. Mesnil suggested that I should investigate the action of these colours on *T. dimorphon* which differs in many ways from the above trypanosomes.

### *Chemistry of the benzidine colours.*

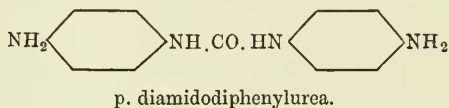
Before proceeding with the question of their action, it will be first necessary to explain briefly the somewhat complicated constitution of these colouring matters.

As regards its chemical constitution, each colour may be considered as consisting of two parts, a base and a side-chain. The base, which is

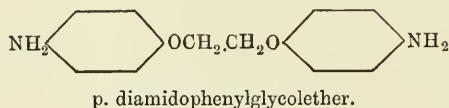
benzidine, or one of its homologues or derivatives, contains two amido ( $\text{NH}_2$ ) groups which occupy the positions indicated in the figure. Benzidine is composed of two benzene rings, united apex to apex, in which two atoms of hydrogen have been replaced by two amido groups. With reference to the point of union of the two benzene rings, substituting groups may enter the nucleus at the ortho, meta, or para (*o*, *m*, *p*) positions. In benzidine the two amido groups have taken up the para position. In other bases, all of which have amido groups in the para position and may be referred to benzidine as their type, other substituting groups are found in the ortho and meta positions.



In some cases the two benzene rings are not directly united to one another, but are joined by an intermediate group. This is the case with the base para diamidodiphenylurea where the group urea

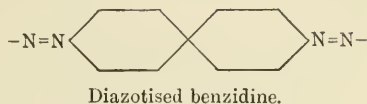


( $\text{NH}_2.\text{CO}.\text{NH}_2$ ) is the connecting link. In another case, the group glycol ( $\text{OH}.\text{CH}_2.\text{CH}_2.\text{OH}$ ) forms the intermediate link, giving rise to the compound para diamidophenylglycolether.



Whatever their constitution, all these bases agree in having the two amido groups in the para positions. These amido ( $\text{NH}_2$ ) groups

may be converted into diazo ( $\text{N}=\text{N}-$ ) groups. With benzidine this would give diazotised benzidine, a compound which only theoretically exists, and which unites directly it is formed with some other group.

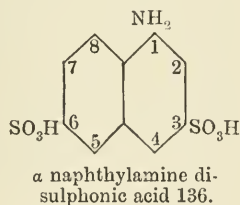


By diazotising benzidine in the presence of one of the side-chains presently to be described, the benzidine may be caused to unite to this side-chain, and thus to give rise to one of the benzidine colours in which there is a benzidine nucleus united to a side-chain in each of its para positions.

The following is a list of the bases which have been employed in these experiments. After each one is placed the abbreviation which is used in the complete list of colours given below.

1. Benzidine (B).
2. Dianisidine (D).
3. Tolidine (T).
4. Dichlorbenzidine (Di.Cl.B).
5. Dichlordianisidine (Di.Cl.D).
6. Benzidineorthomonosulphonic acid (B.o.m.s).
7. Benzidinemetamonosulphonic acid (B.m.m.s).
8. Benzidineorthodisulphonic acid (B.o.di.s).
9. Benzidinemetadisulphonic acid (B.m.di.s).
10. Paradiamidodiphenylurea (P.d.d.u).
11. Diamidophenylglycolether (D.p.g.e).

The side-chains are sulphonic acid derivatives of naphthylamine. It was shown by Nicolle and Mesnil that unless the side-chain contained at least one amido ( $\text{NH}_2$ ) group, and at least two ( $\text{SO}_3\text{H}$ ) groups it would be inactive against trypanosomes. Accordingly, the simplest active side-chain would be one of the type  $\alpha$  naphthylamine disulphonic acid 136. By varying the position of the two ( $\text{SO}_3\text{H}$ ) groups other  $\alpha$  naphthylamine disulphonic acid compounds may be obtained as the types 148, 147, 146, 138, 137, etc. If the amido ( $\text{NH}_2$ ) group be in the 2 position the  $\beta$  naphthylamine disulphonic acids are obtained as the types 237, 236, 257, etc. Similarly occur compounds with two amido groups, the naphthalenediamine disulphonic acids, as the form 1836, which may be considered to be derived from the  $\alpha$  naphthylamine

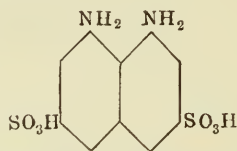




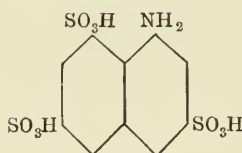
disulphonic acid 136 by the introduction of a second amido group in position 8. There are also  $\alpha$  and  $\beta$  naphthylamine trisulphonic acids as



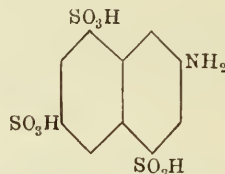
$\beta$  naphthylamine disulphonic acid 237.



Naphthalenediamine disulphonic acid 1836.

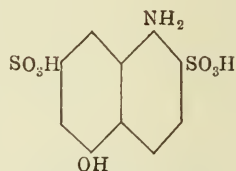


$\alpha$  naphthylamine trisulphonic acid 1368



$\beta$  naphthylamine trisulphonic acid 2468.

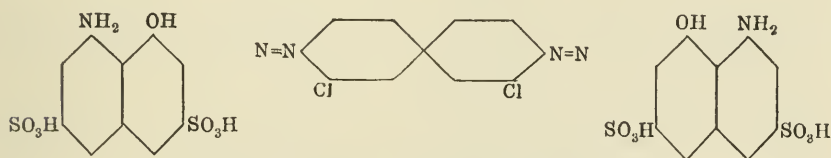
Amidonaphthol disulphonic acids also occur in which a hydroxyl group (OH) has entered the nucleus. Of such a type is the compound amidonaphthol disulphonic acid 1527. Those side-chains which contain other groups besides the necessary one ( $\text{NH}_2$ ) and two ( $\text{SO}_3\text{H}$ ) groups, may be considered as being derived from either the  $\alpha$  or  $\beta$  naphthylamine disulphonic acids by the introduction of these other substituting groups. The realisation of this fact is important, for if the action of a compound of the type  $\alpha$  naphthylamine disulphonic acid 136 is known, and also that of a compound of the type  $\alpha$  naphthylamine trisulphonic acid 1368, any difference in the actions of the two compounds must be due to the fact that in the first, there is wanting the third ( $\text{SO}_3\text{H}$ ) group, which is present in position 8 in the second. As stated above, each colour is composed of a base united to two side-chains. Any one of the above-mentioned bases may be united to any one of the side-chains. In naming these colours the side-chain is named first, and then the base to which it is combined. Thus the colour amidonaphthol disulphonic acid 1836 + dichlorbenzidine is the base dichlorbenzidine united to two molecules of the side-chain amidonaphthol disulphonic acid 1836.



Amidonaphthol disulphonic acid 1527.

In the case of the amidonaphthol disulphonic acids it happens that the union between base and side-chain may be effected in an acid

as well as in an alkaline medium. In other cases the union is only brought about in alkaline medium. When the union can take place in both media, the compound resulting from the union in an acid medium differs from that obtained from a union in an alkaline medium. This difference is due to the fact that in one medium the side-chain unites to the base by one position (position 2 for instance) while in the medium of opposite reaction the side-chain is, as it were, turned completely round, and unites to the base by the corresponding position on the opposite side. For position 2 in one medium it would be position 7 in the other medium. The resulting compounds, though differing so slightly in their chemical constitution, show marked differences in their therapeutic actions.



Amidonaphthol disulphonic acid 1836 + dichlorbenzidine.

In the list of colours given below, where the union between base and side-chains has been in acid medium (ac.) is inserted, and where one side-chain has been united in acid and the other in alkaline medium (ac. alk.) is inserted. In all other cases the union of both side-chains has been in alkaline medium.

These sulphonic acids are not employed as free acids, but chiefly as salts in union with sodium.

For further particulars as to the chemistry of these complex colours readers are referred to the papers by Nicolle and Mesnil (1906) and to treatises on industrial chemistry.

#### *Irregularity of the infection produced by Trypanosoma dimorphon.*

In the treatment of mice infected with *T. dimorphon*, one encounters a difficulty which is not present in the treatment of the infection produced by *T. Brucei*. The strain of *T. Brucei* used by Nicolle and Mesnil gave rise to an infection of such regularity that mice died in exactly three days after trypanosomes appeared in the peripheral blood, so that it was possible to appreciate a survival under treatment of less than 24 hours. *T. dimorphon*, on the contrary, produces in mice an irregular infection which brings about the death of the animal in

any time between two weeks and three months, or even more. Rarely do the trypanosomes disappear from the peripheral blood when once they have been found. When this does occur, usually the trypanosomes are absent only for a few days, while very exceptionally there may be a complete and permanent disappearance of the parasites. These irregularities in the infection compared with that produced by *T. Brucei*, make it much more difficult to judge of the action of any drug, and also, in this instance, render the criterion used by Nicolle and Mesnil impracticable. It is impossible to appreciate any increase in the length of life of the animals, since death takes place at such varying intervals after infection. For this reason I noted the number of days during which trypanosomes remained absent from the peripheral blood after treatment with any colour. This was taken as an index of the activity of the drug in question. The mice were treated on the second day of the infection. The incubation period is very irregular, the average for 121 mice being ten days. In some cases the mice were naturally immune and resisted several inoculations. The duration of the infection is, generally speaking, in proportion to the number of trypanosomes present in the blood. Where multiplication is rapid, and the blood becomes quickly crowded with large numbers of trypanosomes, death will take place more rapidly than in those cases where multiplication is slow. Death appears to be due more to the mass of trypanosomes present than to any toxic influence they may have. With enormous numbers of trypanosomes present in the blood, mice may appear practically normal. They will then usually die quite suddenly after convulsions, which suggest some sudden stoppage to the cerebral circulation. In sections of the brain of these animals the cerebral vessels are seen to be crowded with trypanosomes to such an extent as would quite explain the convulsions and death, apart from any toxic influence. That there is some toxic influence is well shown by the enormous hypertrophy of the spleen that takes place in the more chronic cases. In these the spleen forms a large tumour in the abdomen, and may increase to such a size as to be one-eighth of the weight of the whole body.

*Method of conducting the experiments.*

The manner in which the experiments were carried out was as follows:—Mice were inoculated subcutaneously with blood from an infected animal. The mice were examined from day to day and, on

TABLE I.

Name of colour			No. of mice	<i>T. dinorphon</i>	<i>T. Brucei</i>
$\alpha$ naphthylamine disulphonic acid		148 + B. ...	2	6	0
		148 + T. ...	1	0	0
		147 + B. ...	2	27	1
		147 + D. ...	1	0	0
		147 + T. ...	1	0	0
		146 + B. ...	1	0	0
		138 + B. ...	2	5	1
		137 + B. ...	1	0	0 $\frac{1}{2}$
		136 + B. ...	1	0	0
		168 + B. ...	2	5	0
		168 + D. ...	1	0	0
		157 + B. ...	6	14	14 $\frac{1}{2}$
		157 + D. ...	1	0	0
		237 + B. ...	2	4	1 $\frac{1}{2}$
		237 + T. ...	1	2	0
$\beta$ naphthylamine disulphonic acid		236 + T. ...	1	12	8
		236 + Di.Cl.B. ...	1	0	4
		236 + B.o.m.S. (Trypanroth)	5	14	$\infty$
		236 + B.m.m.S. ...	1	6	0
		236 + B.o.di.S. ...	1	2	1
		236 + B.m.di.S. ...	1	0	0
		257 + B. ...	3	6	5
		257 + T. ...	1	2	0
		1468 + B. ...	2	2	0
		1368 + Di.Cl.B. ...	2	0	1
$\beta$ naphthylamine trisulphonic acid		2367 + B. ...	1	0	2
		2367 + T. ...	1	0	0
Amidonaphthol disulphonic acid		2468 + T. ...	1	0	0 $\frac{1}{2}$
		1527 + B. ...	2	0	1
		1527 + D. ...	1	0	0
		1836 + B. ...	1	0	7 $\frac{1}{2}$
		1836 + D. ...	2	0	14
		1836 + T. ...	2	5	$\infty$
		1836 + T. (ac. alk.) ...	4	0	$\infty$
		1836 + Di.Cl.B. (alk. alk.) ...	4	4	$\infty$
		1836 + Di.Cl.B. (ac. ac.) ...	1	0	5
		1836 + Di.Cl.D. ...	2	5	10
		1836 + D.p.g.e. ...	2	3	11
		1836 + P.d.d.u. ...	4	4	18
		2517 + B. ...	1	0	5
		2517 + T. ...	1	4	1
		1846 + B. ...	1	3	5 $\frac{1}{2}$
		1846 + T. ...	2	5	18
		1824 + D. ...	1	4	3
		2368 + B. ...	1	6	1
		2836 + B. ...	2	1	0
Glycine of amidonaphthol disulphonic acid		1836 + B. ...	2	8	8
		1836 + D. ...	1	0	2 $\frac{1}{2}$
		1836 + T. ...	2	6	3
Acetyl of amidonaphthol disulphonic acid		1836 + D. ...	1	0	0
		2736 + B. (Alpha) ...	4	$\infty$	$\infty$
Naphthalenediamine disulphonic acid		1836 + B. ...	1	0	0
		1836 + D. ...	1	0	0 $\frac{1}{2}$
		1836 + T. ...	2	8	1

the second day of the infection, each mouse received under the skin of the back about 1 c.cm. of a 1% solution of the colour in distilled water. Physiological saline solution was not used, as the salt is liable to cause precipitation of the colour. The dose of colour for a mouse of 15 to 20 grammes weight was usually one centigramme in 1% solution. In the case of Trypanroth the dose was half this amount. With larger mice the dose was increased according to their size. To obtain the best results a careful selection of mice must be made. They should if possible be quite 20 grammes in weight and in healthy condition. Small mice are much more susceptible to the toxic influences of the colours than the larger ones. Where a colour is having a favourable action the mice will often increase in weight, while the reverse is the case with the inactive colours. It may be possible to predict a coming relapse by the continued loss of weight of the animals.

*Treatment of mice by a single dose of colour.*

In Table I will be found a complete list of the colours employed in these experiments. In the first column is given the number of mice used for each colour tested. In the second column is recorded the maximum result obtained in any case, being the number of days

TABLE II.

Name of colour				<i>T. dimorphon</i>	<i>T. Brucei</i>	<i>T. equinum</i>	<i>T. Evansi</i>	<i>T. gambiense</i>	
$\alpha$ naphthylamine disulphonic acid	...	...	157 + B. ...	...	14	14½	15	—	5
$\beta$ naphthylamine disulphonic acid	...	...	236 + B.o.m.S.	...	14	$\infty$	$\infty$	$\infty$	{ 10 (Monkeys) 20 (Rats)
"	"	"	236 + Di.Cl.B.	...	0	4	2	—	—
Amidonaphthol disulphonic acid	...	...	1836 + B. ...	...	0	7½	3	—	—
"	"	"	1836 + D. ...	...	0	14	13	—	—
"	"	"	1836 + T. ...	...	5	$\infty$	$\infty$	$\infty$ ?	—
"	"	"	1836 + T. (ac. alk.)	...	0	$\infty$	$\infty$	$\infty$ ?	10
"	"	"	1836 + Di.Cl.B.	...	4	$\infty$	$\infty$	$\infty$	22
"	"	"	1836 + Di.Cl.B. (ac. ac.)	...	0	5	5½	—	—
"	"	"	1836 + P.d.d.u.	...	4	18	14	20	30
"	"	"	1836 + D.p.g.e.	...	3	11	—	—	26
"	"	"	1846 + T. ...	...	5	18	7	—	—
Naphthalenediamine disulphonic acid	...	...	2736 + B. ...	...	$\infty$	$\infty$	$\infty$	28	8



during which trypanosomes remained absent from the blood after treatment. In the third column are given the results obtained by Nicolle and Mesnil with *T. Brucei*. It will be seen that with only one colour (naphthalenediamine disulphonic acid 2736 and benzidine) was a permanent cure obtained, and this in only one mouse out of four. The great majority of these colours have little or no action, while only five gave results over ten days.

In Table II the colours found by Nicolle and Mesnil to be best for the trypanosomes of Nagana, Surra, Mal de Caderas, and Sleeping Sickness are compared with the results obtained for *T. dimorphon*. It must be remembered that except for *T. dimorphon* the results represent the number of days of survival of the animals, and are not therefore strictly comparable with the results for *T. dimorphon*.

*Action of side-chains and bases and comparison with results  
obtained for T. Brucei by Nicolle and Mesnil.*

Having employed a very large number of colours in their treatment of Nagana, Nicolle and Mesnil were able, by a method of comparison, to arrive at some of the points of chemical constitution which regulate the action of the colours on trypanosomes. One of these laws has already been mentioned. I refer to the necessary presence of at least one ( $\text{NH}_2$ ) group and two ( $\text{SO}_3\text{H}$ ) groups in the side-chain. Another is the method of union of the two benzene rings in the base. Any other method of union of the two rings than by their apices produces an inactive base.

1. *Side-chains.* The side-chains may be divided into families according to the position of the ( $\text{SO}_3\text{H}$ ) groups. Each family contains as its type a side-chain which has one ( $\text{NH}_2$ ) group and two ( $\text{SO}_3\text{H}$ ) groups. All other members of this family have two ( $\text{SO}_3\text{H}$ ) groups in the same position as in the type and the ( $\text{NH}_2$ ) group in the  $\alpha$  or  $\beta$  position; a third ( $\text{SO}_3\text{H}$ ) group, a second ( $\text{NH}_2$ ) group, or an ( $\text{OH}$ ) group may be present also.  $\alpha$  naphthylamine disulphonic acid 157 is the type of one family, while in this same family are also  $\beta$  naphthylamine disulphonic acid 257, and all other side-chains which have the ( $\text{SO}_3\text{H}$ ) groups in the positions 57. The action of  $\alpha$  naphthylamine disulphonic acid 157 being known, any difference in the activity of the  $\beta$  compound must be due to the fact that the ( $\text{NH}_2$ ) group has changed position from  $\alpha$  to  $\beta$ . In this manner all the colours in one family can be compared, and the influence of the sub-

stituting groups arrived at, as any variation in their activity from that of the type must be due to the presence of the other groups. To do this completely a much larger number of colours would have to be tried than are here recorded, but there are sufficient to illustrate some of the points.

*Sulphonic acid groups 5, 7.* The side-chains which have their ( $\text{SO}_3\text{H}$ ) groups in this position give better results for *T. dimorphon* than for *T. Brucei*. The  $\alpha$  naphthylamine disulphonic acid 157 + B. is more active than the  $\beta$  compound, where the amido group has changed from the 1 to the 2 position. This is true as well for *T. dimorphon* as *T. Brucei*. It is not, however, a general rule that the 1 position is better than the 2 position for the amido group. With other arrangements of the ( $\text{SO}_3\text{H}$ ) groups the reverse is the case.

*Sulphonic acid groups 3, 6.* These are generally less active for *T. dimorphon* than for *T. Brucei*. However, the best side-chain for *T. dimorphon*, as also for *T. Brucei*, is in this family. The best side-chain for *T. dimorphon* must be naphthalenediamine disulphonic acid 2736 (see Table I).

The union of the base Di.Cl.B with amidonaphthol disulphonic acid 1836 in acid medium gives a colour which is quite inactive against *T. dimorphon*, though with *T. Brucei* it prolonged life for five days. This same side-chain united with tolidine, first in acid, and then in alkaline medium, is also quite inactive against *T. dimorphon*. Thus the effect of uniting a base to a side-chain in acid, instead of alkaline medium, is to produce a greater diminution of curative power with *T. dimorphon* than with *T. Brucei*.

With  $\beta$  naphthylamine disulphonic acid 236 + T the results for *T. dimorphon* and *T. Brucei* are 12 and 8. If a third ( $\text{SO}_3\text{H}$ ) group is introduced in position 7, as in  $\beta$  naphthylamine trisulphonic acid 2367 + T, the activity is reduced to *nil* for both trypanosomes. Here then the third ( $\text{SO}_3\text{H}$ ) group is a disadvantage, and this is in agreement with Nicolle and Mesnil, who say that the introduction of a third ( $\text{SO}_3\text{H}$ ) group is more an inconvenience than advantage.

The compound  $\beta$  naphthylamine trisulphonic acid 2367 + B gives 0 for *T. dimorphon* and 2 for *T. Brucei*. In the compound naphthalenediamine disulphonic acid 2736 + B one of the ( $\text{SO}_3\text{H}$ ) groups of the trisulphonic acid has been replaced by ( $\text{NH}_2$ ). This change results in a great increase in activity, the colour then giving  $\infty$  for both trypanosomes. This example illustrates very well how a slight change in chemical constitution may be followed by a marked change in

therapeutic action. On the contrary, the introduction of an (OH) group into the nucleus of  $\alpha$  naphthylamine disulphonic acid 136 + B producing amidonaphthol disulphonic acid 1836 + B is no improvement for *T. dimorphon*, but is for *T. Brucei*, while further, the introduction of a second amido group into the same compound, giving naphthalene-diamine disulphonic acid 1836 + B is no improvement, giving 0 for both trypanosomes.

The acetyl derivative of the side-chain amidonaphthol disulphonic acid 1836 is inactive for *T. dimorphon* as it was for *T. Brucei* while the glycine derivative has the same action on both trypanosomes.

*Sulphonic acid groups 6, 8.* These give better results than with *T. Brucei*.

The introduction of a third ( $\text{SO}_3\text{H}$ ) group into the compound  $\alpha$  naphthylamine disulphonic acid 168 + B giving  $\alpha$  naphthylamine trisulphonic acid 1468 + B causes here again a reduction in the activity.

*Sulphonic acid groups 3, 7.* With this arrangement of the ( $\text{SO}_3\text{H}$ ) groups the  $\beta$  position of the amido group is better than the  $\alpha$  position, the contrary of what is found with the 5, 7 positions of the ( $\text{SO}_3\text{H}$ ) groups. This is true for both trypanosomes.

*Sulphonic acid groups 4, 6.* The compound  $\alpha$  naphthylamine disulphonic acid 146 + B gives 0 for both trypanosomes. With the addition of a third ( $\text{SO}_3\text{H}$ ) group in position 8, there is a slightly better action on *T. dimorphon*, this being an exception to the usual rule. The introduction of an (OH) group into the same compound, giving amidonaphthol disulphonic acid 1846 + B, markedly increases the action on both trypanosomes.

*Sulphonic acid groups 4, 8.* The compounds  $\alpha$  naphthylamine disulphonic acid 148 + B and the  $\alpha$  naphthylamine trisulphonic acid 1468 + B again illustrate the unfavourable action of the third ( $\text{SO}_3\text{H}$ ) group.

The other arrangements of the ( $\text{SO}_3\text{H}$ ) groups were not tried in a sufficient number of colours to enable any comparisons to be made.

The best arrangement for the ( $\text{SO}_3\text{H}$ ) groups for *T. dimorphon* in order of merit are 3, 6, 4, 7 (bad for *T. Brucei*), 5, 7 and 4, 8 (bad for *T. Brucei*).

*2 bases.* A complete list of the bases used has been given in the chemical part of this paper.

A comparison between the activities of the different bases may be obtained by comparing their activities when united to the same side-chain. As pointed out by Nicolle and Mesnil, it does not follow that a good base united to a good side-chain will give rise to a good

colour. There exists some relation between the base and the side-chain with which it is combined, that regulates the action of the colour produced. One of the laws which is true for the influence of the base on the side-chain was discovered by Nicolle and Mesnil for *T. Brucei*. It is also true for *T. dimorphon*. If a side-chain with a  $(\text{SO}_3\text{H})$  group in position 6 is tried in combination with benzidine, dianisidine, and tolidine, it will be found that the action on the trypanosomes is greatest in the combination with tolidine, and that it decreases towards benzidine, dianisidine occupying an intermediate position. If, however, the side-chain has a  $(\text{SO}_3\text{H})$  group in position 7 the order of the activities is reversed so that the combination with benzidine has the greatest power, and with tolidine the least. With *T. dimorphon* there is one exception to this law where the result obtained with amidonaphthol disulphonic acid  $2517 + B = 0$  and amidonaphthol disulphonic acid  $+ T = 4$ .

Benzidine orthomonosulphonic acid, and benzidine orthodisulphonic acid are better than the corresponding meta compounds. (This is in combination with  $\beta$  naphthylamine disulphonic acid 236.) Dichlorbenzidine is better than benzidine (in combination with amidonaphthol disulphonic acid 1836). These results are in agreement with those obtained for *T. Brucei*. On the contrary, however, dichlordianisidine is better than dichlorbenzidine for *T. dimorphon*.

#### Comparison with other trypanosomes.

It was found by Nicolle and Mesnil that the best side-chain for the four trypanosomes *T. Brucei*, *T. equinum*, *T. Evansi*, and *T. Gambiense* is amidonaphthol disulphonic acid 1836, which gave an  $\infty$  result (except with *T. Gambiense*). The next is  $\beta$  naphthylamine disulphonic acid, except for *T. Gambiense* and *T. Brucei*, where naphthalenediamine disulphonic acid 2736 is second. This last side-chain is the third in value for *T. equinum* and *T. Evansi*.

In their reaction to the side-chains just mentioned these four trypanosomes show a close agreement. *T. dimorphon* varies considerably in its reaction to these side-chains. For this trypanosome the best side-chain is naphthalenediamine disulphonic acid 2736, and is the only one which gave an  $\infty$  result. Then come the side-chains  $\alpha$  naphthylamine disulphonic acid 147 and 157, the glycine of amidonaphthol disulphonic acid 1836, and finally amidonaphthol disulphonic



acid 1836 itself, which we have seen was the best for the other trypanosomes.

For the four trypanosomes mentioned above the best base is dichlorbenzidine, except that for *T. Gambiense* para diamidodiphenylurea is superior. Tolidine is as good, or nearly so, for *T. Brucei*, but is inferior for the other three.

For *T. dimorphon* dichlorbenzidine is generally a very bad base. In combination with  $\beta$  naphthylamine disulphonic acid 236 benzidine-orthomonosulphonic acid is superior to the other benzidine sulphonic acids as is true for *T. Brucei*.

As regards their reaction to the colours the four trypanosomes, which have just been considered, show marked differences from *T. dimorphon*. The colours which were found to be the most active for these four trypanosomes were blue, while the red colours were inferior to these, though still active. With *T. dimorphon* it was found that the blue colours had usually no action whatever in causing the trypanosomes to disappear, and it often happened that the animals treated died more quickly than if they had been left alone. In one or two cases the trypanosomes disappeared for a day or two after the treatment with a blue colour, but the best colour for the three trypanosomes *T. Brucei*, *T. equinum*, and *T. Evansi*, namely, amidonaphthol disulphonic acid 1836 + Di.Cl.B only gave a result of four days with *T. dimorphon*. The same result was obtained for *T. dimorphon* with the colour, also blue, which was found to be most active against *T. Gambiense* (see Tables I and II). With *T. dimorphon* it was the red colours which were most active, though only one of these gave an  $\infty$  result. This colour, naphthalenediamine disulphonic acid 2736 + B, also gave the same result in the case of *T. Brucei* and *T. equinum*, and was active to a less extent with the other two trypanosomes. It thus appears that *T. dimorphon* as regards its reaction to the red colours is allied to these trypanosomes, but as regards its reaction to the blue colours, is widely separated from them. No reason can be offered as to the cause of this difference between the red and blue colours. As a rule the blue colours are found amongst the amidonaphthol compounds, while the red ones occur amongst the others.



*Treatment of relapses.*

This was not in any way satisfactory. The further treatment of a mouse after having relapsed, though it might cause a second disappearance of the trypanosomes, was no more successful in bringing about a cure than the first treatment. The time during which the trypanosomes remained absent from the blood after the second treatment was always shorter than after the first treatment. Usually a third treatment at the second relapse would kill the mice, but in the few cases that the mice survived a third treatment, a third disappearance of the trypanosomes would take place, and this for a shorter time than either after the first or second treatments. In one mouse treated with Trypanroth a third relapse took place, and a fourth treatment, followed by a fourth disappearance of the trypanosomes, was made. This mouse, however, died, evidently from the toxic effect of the drug, two days later. This diminution in the length of time during which trypanosomes remained absent from the blood as the relapses increased in number, is in agreement with the observations of Nicolle and Mesnil on the trypanosomes with which they worked. They, however, found that in certain cases after a number of relapses and treatments, the colour became quite inactive against the trypanosomes.

In the treatment of the relapses of *T. dimorphon* the colour employed was always the same as had been given at the first treatment. It might be possible to obtain better results by using for the second treatment a different colour. No attempt was made to treat the relapses unless five days had elapsed since the first treatment. If trypanosomes were found to have reappeared in the blood on the sixth day, half the initial dose of colour was at once administered. This dose was increased with the interval of time from the first treatment.

*Treatment by repeated doses of colour.*

With single doses of colour, we have seen that only with the colour naphthalenediamine disulphonic acid 2736 + B did a permanent cure result. With four other colours results over ten days were obtained (see Table I). With these five colours attempts were made to treat the mice by giving repeated doses of colour so as to prevent, if possible, the relapses. For each colour three mice were used. In one series treatment was made on the 2nd, 5th, 8th, and 11th days of the infection, in another series on the 2nd, 7th, and 12th days,

TABLE III.

Name of colour	No. of experiment	Days of treatment	Doses on corresponding days (centigrams)	Results
$\alpha$ naphthylamine disulphonic acid 147 + benzidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Relapse 21 days after 1st treatment.
	2	2, 7, 12	1·3, ·5, ·5	" 19 " " "
	3	2, 10	1·2, ·8	" 5 " " "
$\alpha$ naphthylamine disulphonic acid 157 + benzidine	1	2, 5, 8	1·2, ·3, ·3	Died without relapse 7 days after 1st treatment.
	2	2, 7, 12	1·2, ·5, ·5	" " " 21 " " "
	3	2, 10	1·2, ·8	" " " 14 " " "
$\beta$ naphthylamine disulphonic acid 236 + toluidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Died without relapse 12 days after 1st treatment.
	2	2, 7, 12	1·0, ·5, ·5	" " " 14 " " "
	3	2, 10	1·2, ·8	" " " 13 " " "
$\beta$ naphthylamine disulphonic acid 236 + benzidine orthomonosulphonic acid (Trypanroth)	1	2, 5, 8, 11	·5, 1·5, 1·5, 1·5	Relapse 14 days after 1st treatment.
	2	2, 7, 12, 17	·6, ·25, ·25, ·25	" 24 " " "
	3	2, 10, 18	·6, ·4, ·2	No relapse.
naphthalenediamine disulphonic acid 2736 + benzidine	1	2, 5, 8, 11	1·2, ·3, ·3, ·3	Died without relapse 14 days after 1st treatment.
	2	2, 5, 8, 11	1·2, ·3, ·3, ·3	No relapse.
	3	2, 7, 12	1·2, ·5, ·5	" " " "
	4	2, 10, 18	1·2, ·8, ·4	" " " "

and in the last series on the 2nd and 10th days. The details of the treatment are given in Table III. The second and third colours in this table caused such extensive necroses of the skin that all the mice died from this cause without having relapsed. The fourth colour (Trypanroth) was able to bring about a permanent cure in the third mouse. With the last colour all the mice were cured with the exception of the first, which died from the toxic effects of the colour. This last colour, which was the one which gave an  $\infty$  result with the single dose, is evidently superior to the other colours as far as its action on *T. dimorphon* is concerned.

*Other strains of T. dimorphon.*

To further test the results obtained with the colour naphthalene-diamine disulphonic acid 2736 + B, two other strains of *T. dimorphon* were used. These were very kindly given me by Dr Gustave Martin, who had brought them from French Guinea. One strain was from a pig and the other from a dog. In each case the colour brought about a permanent disappearance of the parasites.

*Harmful influence of the colours on mice.*

In treating mice with these colours two difficulties have to be encountered, their toxicity and their property of causing local necrosis. The latter can, to some extent, be avoided by careful inoculations, but in spite of all precautions, some of the colours will cause sloughing of large areas of skin. It is probable that this is more readily produced in infected than in uninfected mice and also, what at first sight is not so apparent, that where the drug has a curative action, it is not so likely to occur.

The toxicity of the colour causes the death of the animals with or without any local necrosis. If a large dose is given the animals may die in a few hours. With smaller doses death does not take place for two or three days, and it may be after the complete disappearance of the trypanosomes. As shown by Dr Bouffard (1906), the kidney is one of the three places in the body where the colour is deposited in the form of granules. In sections of the kidney of some of my mice which had died from the toxic effects of the drugs, the cells of the convoluted tubules were packed with granules of the colour, and many of them were in a condition of necrosis. It is probable that in these cases the mice had died from the nephritis. Fortunately, as regards

both these dangers, the colour found to be most active against *T. dimorphon* is very well tolerated by the mice.

*Concluding remarks.*

As regards the changes produced in the trypanosomes themselves by treating mice with these colours, there is nothing more to add to the description given by Nicolle and Mesnil. Soon after the injection of the colour, distorted forms of trypanosomes appear in the blood. The proportion of these forms increases, and finally the trypanosomes disappear completely from the blood if the colour is sufficiently active. What becomes of the trypanosomes between the time of their disappearance and the relapse is not known. It is a question allied in many ways to the relapses in some diseases, for instance in relapsing fever, where the disappearance of the parasites from the blood is probably due to some substance present in the blood, which is active against these parasites. As regards the benzidine colours, a glance at Tables I and II is sufficient to show that *T. dimorphon* is more resistant than the other trypanosomes. This is in conformity with the observations of other workers. Laveran, working with human serum, found *T. dimorphon* more resistant than the trypanosomes of Nagana, Surra, and Mal de Caderas, though with trypanosomes fairly numerous in the blood, a sufficient dose of human serum would cause their temporary disappearance. Thomas and Breinl (1905) found *T. dimorphon* "harder to combat with atoxyl, or any other form of arsenic," than the other trypanosomes mentioned in this paper. These authors found that "animals infected with *T. dimorphon* do not react well to arsenical treatment by itself; Trypanroth medication only causes the parasites to temporarily disappear; the combination of arsenic and Trypanroth causes the parasites to be absent for a longer period." In testing the action of atoxyl and arsenic I obtained for the former a result of only two days, and for the latter, in the form of sodium arsenite, a result of six days<sup>1</sup>.

*T. dimorphon* has thus proved more resistant to all forms of medication that have hitherto been tried, than other trypanosomes. The only drug which promises success being the naphthalenediamine disulphonic acid 2736 + benzidine (named alpha). So far this colour has only been tried in mice and here only gives successful results when given in repeated

<sup>1</sup> See p. 280, *et seq.* This refers to the number of days during which trypanosomes remained absent from the blood after treatment.

doses. It still awaits trial in larger animals, where it is hoped that still more successful results may be obtained.

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# ON THE LARVAL AND PUPAL STAGES OF ANOPHELES MACULIPENNIS, MEIGEN.

(Plates IV—V, 1 Text-Fig.)

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## PART I. THE LARVA.

THE great importance of an exact knowledge of the etiology of malaria has given a remarkable impetus to the study of mosquitoes. It has resulted in the growth, during the last few years, of a very extensive literature relating to these insects, and especially to those of the genus *Anopheles*, which serve as the alternative and definitive hosts of human malarial parasites. Comparatively few writings, however, deal with their internal anatomy.

In the present instance an attempt is made to describe the internal structure of the larva and pupa of *Anopheles maculipennis*, concerning which no adequate account yet exists. I am indebted to Mr A. E. Shipley for suggesting to me this line of investigation, and for placing at my disposal a large number of microscopical sections. Prof. G. H. F. Nuttall has also generously assisted me during its progress. The work has been carried out in the Morphological Laboratory at Cambridge during a tenure of an 1851 Exhibition Scholarship.

## THE LARVA.

For the purposes of study larvae were collected in the neighbourhood of Cambridge during the year 1906. They were met with on various dates, ranging from May 9th up to August 10th, and were obtained from the following four localities. (1) A small bricked excavation in the middle of a field; it measured one and a half feet in cubical dimensions. In addition to larvae of *Anopheles*, the

water contained *Cyclops*, green larvae of *Chironomus*, a few small Coleoptera and confervoid Algae. (2) A roadside pond partly covered with *Lemna*, and containing great numbers of Ostracoda, together with red larvae of *Chironomus*. (3) Still places in the backwaters of the Cam. (4) Two ponds situated in a field. There were no trees near them, and their banks were fringed with a good deal of miscellaneous herbage. In one of the ponds they were met with in company with larvae of *Dixa*, *Chironomus*, and of an undetermined species of Psychodidae. The pond also contained numerous dragon-fly larvae belonging to several species, together with may-fly larvae and a number of small fish (measuring up to about eight inches in length). In the other pond there were no fish but great numbers of dragon-fly and may-fly larvae, and also larvae of the Stratiomyid fly *Odontomyia*. Both ponds contained an abundance of various Hemiptera and small Coleoptera.

The *Anopheles* larvae were scarce during the whole period, and although they were sought for elsewhere none were discovered in any other localities around Cambridge.

The external morphology of the larva is now comparatively well known. Good descriptions and figures have been given by Meinert (1886, p. 373), Grassi (1900), Howard (1901), and Nuttall and Shipley (1901, p. 45). The account given by the two latter authors is the fullest and most satisfactory and, moreover, is accompanied by the best and most detailed figures yet published. In the present paper only a brief description of the general external features of the *Anopheles* larva will be given.

#### *External Form.*

The larva may be divided into three distinct regions: (1) the *Head*, (2) the *Thorax*, (3) the *Abdomen* (Fig. 1)<sup>1</sup>.

The *Head* is invested by a well-defined brown chitinous capsule. Its diameter from above downwards very nearly equals that taken from side to side (Fig. 2). At its anterior end it carries a pair of bunches of dark brown hairs; they are termed by Nuttall and Shipley the "brushes"<sup>2</sup>. The movements of these organs in the vertical plane set up a current in the water and by this means particles of food are wafted towards the mouth. These brushes are borne on a median anterior area termed by Meinert the "clypeus." It seems probable that this sclerite should be regarded as an undifferentiated clypeo-labrum.

<sup>1</sup> For Figs. 1—20 see Plate IV, for Figs. 21—33 see Plate V.

<sup>2</sup> By Blanchard and other writers they are termed the rotatory organs.

Stretching transversely across the middle of the head is a dark band of pigment, and over this area is disposed a row of six feathered hairs. A stout branched hair is placed immediately above, and overhanging each brush. Between these two hairs, and lying very close together on either side of the median line, are a pair of simple bristle-like hairs, slightly branched at their extremities (*fr.h.* in Fig. 1). This row of four frontal hairs has been shown by Grassi to have significance as a specific character<sup>1</sup>, and he figures their differences in *Anopheles claviger* (*maculipennis*), *A. bifurcatus*, *A. (Myzomyia) superpictus* and *A. (Myzorrhynchus) pseudopictus*.

The paired appendages of the head consist of the antennae, the mandibles, and the first maxillae. Between the two maxillae there lies a pointed toothed plate. It is termed by Meinert the "under lip" and by Felt (1904) the "labial plate"; the latter writer figures it for the larvae of a large number of N. American Culicidae. This sclerite is most probably to be regarded as the representative of the second maxillae which have united to form a single organ. A similar plate is often found among Dipterous larvae, and usually possesses a second sclerite lying immediately above it. This is the case in *Chironomus*, *Dicranota*, *Phalacroceras*, and *Pericoma*. The lower plate is regarded by Miall and Hammond (1900) as the "submentum" and the upper one as the "mentum." They believe that the mentum has gradually slipped behind the submentum so that the latter completely hides it when viewed from below. In *Anopheles*, the "labial plate" corresponds with the "submentum" of the *Chironomus* larva; it bears on its upper, or pharyngeal surface a considerable array of strongly chitinated teeth and ridges, but I have not been able to homologise any of them with tolerable certainty with a "mentum." A strongly-defined median area is also present and carries the orifice of the salivary duct; it, therefore, is to be regarded as the hypopharynx (Fig. 5).

The larval eyes are situated one on either side of the head, a short distance behind the antennae. Slightly anterior and dorsad of the larval eyes lie the primordia of the compound eyes of the imago. They consist of a variable number of isolated elements (ommatidia), which increase as the larva approaches the pupal period.

The *Thorax* shows externally only very slight indications of a separation into the three characteristic segments. Over its surface are

<sup>1</sup> Theobald (*Monograph of the Culicidae*, Vol. III. p. 15) states that this character has proved of great value.

distributed several groups of feathered hairs (Plate IV, Fig. 1). Lying immediately behind the most anterior row of these hairs on either side, and overhanging their bases, is a curious flattened notched process (*prc.* in Fig. 1) described by Nuttall and Shipley. As to the significance of these organs little can be said. I would suggest that possibly they have been derived from a pair of prothoracic spiracles which, although now lost in the Culicidae, are present in the larvae of the allied family of the Psychodidae.

The *Abdomen* consists of nine segments. The first two segments carry a pair of feathered hairs on each side. The third segment carries a single one on either side, and in the succeeding segments these hairs lose their pinnations, become smaller in size, and reduced to naked bristles. Situated on the dorsal aspect of each segment from the third to the seventh, inclusive, is a pair of small conically branched hairs, the palmate hairs of Nuttall and Shipley (*A.* in Figs. 1 and 2). Each palmate hair consists of a stalk carrying at its apex a whorl of flattened lanceolate leaflike branches. Other hairs are present on these segments but these two types are the most important. Both Stephens and Christophers (1902) and James and Liston (1904), in their accounts of the Indian species of *Anopheles*, show that the number of the palmate hairs, and the shape of each leaflet, afford useful specific characters. The eighth segment carries on its dorsal side the two spiracles, and has undergone modification for this purpose, a rather complex skeleton being developed for the support of those structures. The ninth abdominal segment contains the anus (*an.* in Fig. 3), and around that aperture are arranged two pairs of tracheal gills (*a.g.*). At the hinder margin of the segment, and overhanging the anus, are placed four very long and curved feathered hairs. Ventrally, the segment carries on each side a row of nine long hairs, also of the feathered type, the two rows arising from a common chitinous base.

#### *Note on its Habits.*

None of the numerous figures which occur in literature are strictly accurate as regards the method by means of which the larva attaches itself to the surface film. Nuttall and Shipley remark (1901, p. 57) that "the larvae lie with the long axis of their bodies parallel with the surface of the water.... Viewed from the side, the respiratory apparatus, as also the palmate hairs upon the dorsal surface of the abdominal segments, are seen to indent the surface film. The palmate



hairs just referred to produce a series of minute bilateral indentations in the film, making it appear, on superficial observation, as if the dorsal surface of the larva actually protruded above the surface." After an examination of living larvae, I am able to confirm this observation. On referring to Fig. 2 it will be further noted that while the larva remains just beneath the surface film, the ventral side of its head lies uppermost, the head having been rotated through an angle of  $180^{\circ}$ . In this attitude it can readily use its brushes to set up a current in the water. This has the effect of sweeping towards the mouth whatever small organisms and débris may be floating immediately under the surface film. The movement of the brushes can be readily observed when a larva is transferred, with a drop of water, on to a microscopical slide, and a cover-glass placed very lightly over it. In its efforts to free itself the brushes can be seen to be in active movement, and the head will occasionally be seen rotating on its neck. This latter movement is performed with remarkable rapidity and ease.

#### *The Integument.*

The integument consists of a chitinous cuticle and, underlying it, the hypodermis or chitogenous layer.

The *Cuticle* is smooth and transparent. In the region of the head it is thicker than elsewhere and is seen to be bright yellow in thin sections. Over other parts of the body, except in a few localised places, it is quite colourless. It consists of two layers; an outer and much thinner but highly refractive layer, and a relatively thick inner stratum, which is much softer, and apparently only partially chitinised (Fig. 9). The inner layer is much the more readily stainable of the two. In prepared sections the cuticle is sometimes seen to have split at the junction of these two layers during the process of cutting. In several larvae only the outer layer was found to be present, the inner stratum having been absorbed. This was found to be the case prior to ecdysis and in the narrow space once occupied by the inner layer of the cuticle the hypodermis was seen to be secreting a new layer of chitin on its outer surface.

The *Hypodermis* consists of a single layer of cells resting internally on a delicate basement membrane. Its cellular nature is best seen towards the middle of each of the segments, especially on their dorsal aspect (Fig. 8). In the head, and at the sides and junctions of the segments, the hypodermis appears as an undifferentiated stratum of



protoplasm containing scattered flattened nuclei which are, as a rule, hard to detect (Fig. 7). Distributed through the hypodermis in various places are some enormously enlarged cells (Figs. 12 and 13). These are trichogenous cells, which secrete the feathered and palmate hairs already referred to. They are pyriform in shape and, on account of their size, bulge through the hypodermis into the body-cavity. They contain a large round nucleus with a very prominent nucleolus, and around the latter are arranged threads of chromatin. Those which secrete the palmate hairs are smaller than those which secrete the other type. The row of four hairs which overhang the anus have the largest cells at their bases, and in transverse sections taken through that part of the body these cells form very striking objects, each one nearly equalling the rectum in diameter! In favourable sections a fine thread has been observed to pass into the base of each palmate hair; most probably this is a nerve fibre (*n'*. in Fig. 12).

Excepting in the head region, there occur in many places immediately beneath the hypodermis, and adherent to its inner surface, a layer of flattened irregularly shaped cells (Fig. 8)—the *sub-hypodermal cells* of Viallanes (1882, p. 12). Very frequently several of these cells are united together, and in their protoplasm is distributed a quantity of greenish granular particles. A similar tissue to the above is present in the larvae of *Musca*, *Eristalis* and *Chironomus*. In the latter case, Miall and Hammond (1900, p. 38) conclude from the various stages of aggregation which these cells exhibit, and from their slow changes of figure, they can move from place to place and, that however they may be scattered, they retain the power of combining into an epithelium. They regard them as being the wandering cells (*Wanderzellen*) of Metschnikoff and Kowalevsky. Viallanes states that these cells in *Musca* and *Eristalis* undergo changes analogous to those of the fat-body during the transition from the larva to the pupa. Their protoplasm contains spherical granules which increase in size and number as the larva gets older.

#### *The Digestive System.*

The *mouth-parts* together enclose a space or chamber, at the posterior end of which is situated the mouth itself. The epipharynx forms the roof of this chamber, the mandibles form the sides, and the maxillae, together with the labial plate, the floor (Fig. 4). The side walls are further completed by the overlapping of the brushes on either

side, and also by the prominent hairs on the mandibles which meet those of the brushes.

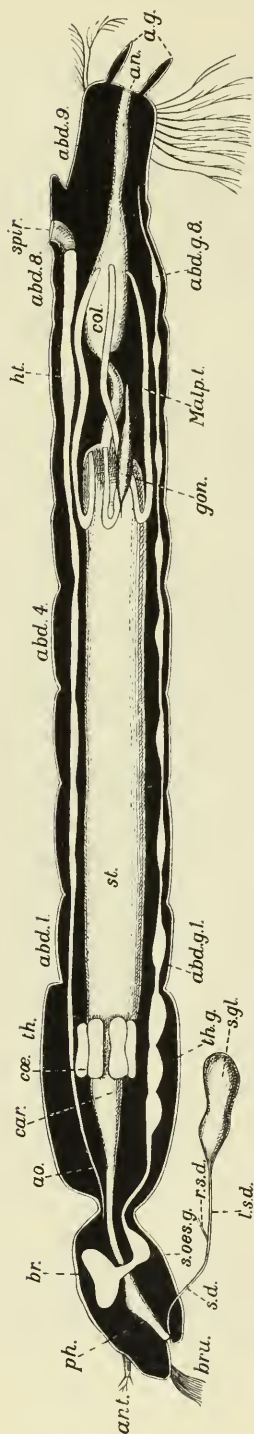
The epipharynx is provided with groups of setae (Fig. 5), and the hypodermis underlying them has a markedly columnar structure. Very possibly they are to be regarded as being of a sensory nature and perhaps gustatory in function. The hypopharynx and the anterior surface of the labial plate have a strong armature of chitinous tooth-like projections.

*The Fore-Gut*.—The fore-gut is divisible into pharynx and oesophagus.

The *Pharynx* is a capacious chamber situated in the anterior half of the head (Fig. 5). Its walls consist of a chitinous intima, continuous with the general cuticle of the body-wall, and resting on a layer of epithelium. Outside the epithelium is a strongly developed layer of muscles (Figs. 10 and 11).

At its commencement, the pharynx is much flattened in the dorso-ventral plane and has, moreover, a greater transverse diameter than in the succeeding portion. It is somewhat crescentic in shape when viewed in section owing to the development of a pair of lateral crests (Fig. 10). A little further backwards, its transverse diameter narrows but, on account of the increased development of the lateral crests, its crescentic shape becomes intensified. At the hinder part of the pharynx the crests become less prominent and, converging, eventually fuse with one another in the median dorsal line, and just in front of the commencement of the oesophagus. The chitinous intima of the roof of the pharynx, between the two crests, becomes considerably thickened on either side and forms a pair of rigid plates (*c.p.*): along the middle line between these plates the intima still remains flexible. Traced posteriorly the plates narrow very much, and terminate at a point just in front of the union of the two lateral crests of the pharynx. On the outside of each chitinous plate, and situated on the crest of its side, is a rod-like thickening of the intima (*rd.* in Figs. 10 and 11), which serves to give a firmer attachment to certain of the pharyngeal muscles. Situated in the crests of the pharynx is a dorsal and ventral longitudinal row of firm bristle-like setae (*s.* in Figs. 10 and 12).

The pharynx is provided with an elaborate musculature. These muscles have been found to agree in their general arrangement with the pharyngeal muscles of the larva of *Culex* as described by Thompson (1905, p. 145), and as most of the names given to them by that



Diagrammatic Figure showing the general organization of the larva of *Anopheles maculipennis*.  $\times$  circa 20.

<i>ant.</i>	antenna.	<i>coe.</i>	caeca.
<i>ao.</i>	aorta.	<i>ht.</i>	heart.
<i>bru.</i>	brush.	<i>l.s.d.</i>	left salivary duct.
<i>br.</i>	brain.	<i>r.s.d.</i>	right do.
<i>abd. 1.</i>	1st abdominal segment.	<i>s.d.</i>	main do.
<i>abd. 4.</i>	4th do.	<i>s.gl.</i>	left salivary gland.
<i>abd. 8.</i>	8th do.	<i>s.oes.g.</i>	sub-oesophageal ganglion.
<i>abd. 9.</i>	9th do.	<i>ph.</i>	pharynx.
<i>abd.g. 1.</i>	1st abdominal ganglion.	<i>Malp.t.</i>	Malpighian tube.
<i>abd.g. 8.</i>	8th do.	<i>gon.</i>	gonad.
<i>a.g.</i>	anal gills.	<i>st.</i>	stomach.
<i>an.</i>	anus.	<i>spir.</i>	spiracle (right).
<i>col.</i>	colon.	<i>th.</i>	thorax
<i>car.</i>	cardia.	<i>th.g</i>	thoracic ganglia

observer are convenient and appropriate, they are adopted in the present description.

The principal pharyngeal muscles are as follows :—

A series of *dorsal pharyngeal muscles*; these are transversely placed fibres stretching between, and uniting the lateral crests of the pharynx ( $m_4$  in Figs. 6, 10 and 11).

A pair of *lateral pharyngeal muscles*; they arise from the integument of the head, close to the point of origin of the antenna on either side, and slightly to the inside of that appendage. They cross the head-cavity obliquely, and become inserted on the summits of the pharyngeal crests ( $m_3$  in Figs. 6 and 11).

A paired series of *anterior lateral pharyngeal muscles* (lesser lateral muscles of Thompson). These consist of four or five slender muscles on each side, each group arising from the antero-lateral border of the head just in front of the antenna of its side. They pass directly inwards in a horizontal fashion, and are inserted into the wall of the pharynx on either side, near to its commencement ( $m_2$  in Fig. 6).

A pair of stout *epipharyngeal muscles*; these arise from the roof of the head a little anterior to the brain. They pass forwards, above the pharynx, at first nearly parallel with one another, but eventually converge to become inserted close together, about the point where the roof of the pharynx becomes continuous with the epipharynx ( $m_1$  in Figs. 5 and 6).

A pair of *longitudinal pharyngeal muscles* (not described by Thompson in the *Culex* larva). These are a pair of slender muscles placed closely together, and lying in the dorsal trough of the pharynx between its lateral crests and beneath the dorsal pharyngeal muscles ( $m_5$  in Fig. 11).

A pair of *elevator muscles* arise from the roof of the head a short distance in front of the brain. They pass almost directly downwards, and are inserted closely together on the roof of the pharynx near to the point where the two lateral crests become confluent ( $m_6$  in Fig. 6).

A pair of *retractor pharyngeal muscles* arise from the hind wall of the head not far from the occipital foramen. They pass to the posterior margin of the pharynx to be inserted a little way behind the points of attachment of the elevator muscles ( $m_9$  in Fig. 6).

A pair of *diagonal muscles*; these muscles arise from the posterior part of the head somewhat external to the retractor muscles. They pass very obliquely forwards and cross one another as they do so, and in this way they become attached to the posterior part of the



pharynx a little in front of the retractor muscles. The point of attachment of each diagonal muscle is situated on that side of the pharynx opposite to its point of origin ( $m_8$  in Fig. 6).

A pair of groups of *lateral dilator muscles*; these are attached to the side walls of the posterior portion of the pharynx, and the immediately adjacent portion of the oesophagus. Each group consists of a bundle of very slender muscles, and they take their origin from the integument of the ventro-lateral region of the head ( $m_7$  in Figs. 6 and 11).

The walls of the pharynx contain a series of circular muscle fibres which are principally developed in relation to its posterior portion. Over the anterior part of the pharynx they are restricted to its ventral and lateral walls (Figs. 5 and 6).

The food is carried into the mouth in the current of water set up by the rapid motion of the brushes and, after being masticated to some extent by the action of the mandibles and maxillae, is passed into the pharynx. It is not possible, however, to make much out as regards the actual working of the pharyngeal muscles in the living larva on account of the want of transparency of the integument of the head. It appears to take place somewhat as follows. When the pharynx becomes filled with food a contraction of its dorsal muscles take place, resulting in the drawing together of the two pharyngeal crests, and causing the two dorsal chitinous plates to form an acute angle with each other, and to come very nearly in contact with its floor. The action of these muscles is assisted by that of the circular and diagonal muscles, and the result of their combined action is that the pharyngeal cavity becomes greatly reduced and much altered in its shape. The effect is the forcing of the food backwards into the oesophagus, and constituting the act of swallowing. The lateral, elevator, and retractor muscles most probably serve to assist in bringing about the divergence of the pharyngeal crests after swallowing, but very likely fulfil other functions also. At the junction of the pharynx with the oesophagus the food is propelled backwards by means of the lateral dilator and circular muscles, and afterwards the work is carried on entirely by the muscles of the oesophagus.

The *Oesophagus* is a narrow tube of simple structure (Figs. 3 and 16). Its innermost coat is a well-defined chitinous intima, the middle coat is an epithelium, and the outermost is muscular. The cellular nature of the epithelium is usually only to be discerned by the presence of nuclei. The muscular coat consists of a strongly developed layer of



circular fibres which are markedly striated. Each muscle band is in contact with its fellows, and in this way the muscular coat forms a complete investment to the oesophagus (Fig. 25). They increase in thickness as they are traced backwards, and attain their maximum in the oesophageal valve described below. Near to the junction with the pharynx, several radial muscle fibres pass outwards between the circular bands, and are attached to the walls of the head; they function as the dilators of the oesophagus. Outside the muscular coat is a thin membrane of connective tissue. The lumen of the oesophagus alters in shape a good deal in different parts, and is much reduced by inwardly directed bulgings, which involve both the cuticular and epithelial layers.

Followed backwards into the middle of the thorax, the oesophagus joins the cardia, or first division of the mesenteron (Fig. 3). It is prolonged into the cavity of the latter for some distance as an inner tube and then becoming reflected on itself, passes forwards again to unite with the epithelium of the mid-gut (Fig. 25); in this way there is formed an oesophageal or cardiac valve. Both the chitinous intima and the epithelium of the fore-gut are thus reflected, but the circular muscle bands cease some distance before the bend is reached: some stout longitudinal fibres are also present. The space between the inner or downwardly directed wall and the outer or reflected wall of the oesophagus contains a good deal of blood, and there is also a circular sinus situated between the muscular layer and the inner wall (Fig. 25). This structure surrounds the gut in the form of a complete ring; it is covered by a layer of connective tissue, and its interior shows a spongy structure, its cavity being much intersected by strands of a fibrous nature. It has been found in several Dipterous larvae, and is regarded as being a blood sinus. A fibrous band passes from the oesophagus across to the shoulder or apex of the cardia (*lig.* in Fig. 25): it contains no muscle fibres, and in all probability functions as a ligament. It appears to be derived from the outer connective tissue coat of the oesophagus, and it passes into that of the cardia.

An oesophageal valve seems to be of very general occurrence among the larvae of Diptera, and it exhibits a fairly wide range of complexity. In the *Dicranota* larva it is very simple, and the oesophagus only protrudes very slightly into the mesenteron. In *Culex* it is in the same condition as in *Anopheles*. In *Simulium*, according to the figures given by Miall and Hammond (1900, p. 61), the circular muscles are more strongly developed, and extend close down to the apex of the valve, the blood sinus is much larger and, moreover, differs from that of *Anopheles* in being placed between the layer of circular muscles and the outer or reflected wall of the valve. In *Chironomus*, according to those observers, the valve has

the same general arrangement as in *Simulium*, but is complicated by secondary foldings of the walls of the oesophagus, which involve both the intima and the epithelium, and are termed by them the upper and lower intermediate bands. In the *Ptychoptera* larva the valve attains a remarkable complexity of structure. According to van Gehuchten (1890, p. 185) there are secondary foldings as in *Chironomus*, and there is a special sphincter muscle situated at the commencement of the valve, in addition to the usual circular fibres. The blood sinus is very extensive, and its cavity is traversed by numerous strands, some of which are muscular and others elastic. There is further, a secondary folding in the wall of the cardia forming a circular "proventricular valvule" (van Gehuchten, Plate 1, Fig. 5 and Plate 3, Fig. 44).

*The Salivary Glands*:—The salivary glands are situated in the thorax one on either side of the digestive canal. Each gland is a hollow vesicle lined by a single layer of cells (Fig. 22), and passing at its proximal end into a narrow duct (Text-Fig. p. 298). The two ducts unite within the head near to the sub-oesophageal ganglion, and the unpaired channel thus formed opens into the mouth on the dorsal aspect of the labial plate in the median line (*ap.* in Fig. 5). In the very young larva the salivary glands are relatively of less bulk as compared with those of full-grown examples. The structure of the glands is extremely simple, each consisting of a layer of epithelial cells, bounded on the exterior by a thin coat of connective tissue. The cells are very large and are polygonal; they bulge inwards to some extent into the lumen of the gland. Their nuclei are extremely prominent (Figs. 22 and 23), and they each contain a large nucleolus, together with thick loops of chromatin. The ducts are very thin walled, and are lined with a delicate intima: the median duct just before opening on to the hypopharynx expands into a slightly bulbous enlargement. No "spiral thread" could be detected though it is found in the salivary ducts of the adult mosquito.

*The Mid-gut or Mesenteron*:—The mid-gut is a long cylindrical tube extending from the middle of the thorax nearly to the posterior border of the sixth abdominal segment, where it joins the hind-gut (Text-Fig.). Throughout the greater part of its course it is of a uniform calibre, but it narrows somewhat near its posterior end. It is separable into three divisions, viz. (1) the *Cardia*, (2) a circlet of *eight caecal pouches*, (3) the *Stomach*.

The *Cardia* is that portion of the mid-gut which encloses the oesophageal valve (Figs. 3 and 25). I have followed Miall and Hammond in using this term in preference to the name *proventriculus* which is employed by many authors. The word *proventriculus* has long been

associated with the gizzard in Insecta which is derived from the stomodaeal ectoderm. Miall and Hammond have demonstrated by a study of the embryological development of *Chironomus* that the wall of the cardia is produced from the endoderm. There is every reason to believe that the same is the case in *Anopheles*, and many other Dipterous larvae. This is supported by the fact that the cells of the cardia have the same general character as those lining the other parts of the mid-gut. The break where the epithelium of the oesophagus terminates, and that of the cardia commences, is clearly marked (Fig. 25), and the cardiac chamber of the stomach may be regarded as extending from this point backwards to where the eight caeca are given off from the mid-gut. The epithelium of the cardia is, for the most part, composed of large and markedly columnar cells, which stain more deeply than those of any other part of the mesenteron. Their nuclei are large and very prominent, and contain a good deal of chromatin in the form of coiled threads (Fig. 25). Further backwards, however, the epithelium instead of being columnar becomes cubical, and then passes by a gradual transition into that lining the caeca. The cardia is invested exteriorly by a coat of connective tissue; there are no muscle fibres present in its walls.

The *Caeca* are eight sub-equal diverticula of the mesenteron arranged in the form of a ring or circlet (Figs. 3 and 26). Each caecum is constricted across its middle, as if it were bound by a circular ligament (Fig. 25). Nothing, however, of such a nature is present and, moreover, the caeca contain no muscle fibres in their walls. The epithelial lining consists of rather larger cells than those of the stomach, and they bulge somewhat into the central cavity (Fig. 18). Externally they rest on a membrane of connective tissue. The caeca are filled with a thin dark yellow fluid which is coagulable by alcohol (Figs. 18 and 25). At times the free portions of the cells bordering the cavities appear swollen by a transparent fluid secretion, but in no instance were they observed to give off protrusions as the cells of the stomach frequently do.

These caeca are of frequent occurrence among Dipterous larvae, and vary very considerably in size and number. In the larvae of both *Culex* (Raschke), and *Ptychoptera* (Gehuchten), eight caecal diverticula are present as in *Anopheles*. In those of *Tipula* (Hammond), *Piophilus* (Dufour), *Simulium* (Miall), *Anthomyia* (Vaney), *Volucella* (Künckel d'Herculais) and *Calliphora* (Lowne), there are four caeca. In *Sciara* (Packard), *Ceroplatus* (Dufour) and *Sapromyza* (Dufour), there are only two, but in these instances they are very long and tube-like. In *Chiro-*

*nomus* the caeca are small and very numerous, and arranged in three sets (Miall and Hammond). Caeca are wanting, however, in *Phalacroceras* (Miall and Shelford), *Diceranota* (Miall) and *Psychoda* (Dell).

With regard to the function of these organs, it may be mentioned that Hoppe-Seyler, Plateau, Krukenberg, and others maintain that the digestive properties of the already mentioned fluid contained within them are of a similar nature to that of the pancreas in vertebrates.

The *Stomach* comprises the whole of the remaining portion of the mesenteron (Fig. 3 and Text-Fig.). Its walls consist of the following, passing from within outwards:—

1. The peritrophic membrane (*p.m.* in Figs. 14 and 15). It commences on the cardiac epithelium close to the junction of the latter layer with that of the oesophagus. This membrane invests the whole of the inner surface of the mesenteron, excepting that it is not reflected into the cavities of the caeca.

2. The epithelial layer, which is composed of columnar polygonal cells with prominent rounded nuclei. Each nucleus contains a sharply defined nucleolus, together with a number of large chromatic granules (Fig. 14).

3. The basement membrane; it is a well-developed coat and stains readily (*b.m.* in Figs. 14 and 15).

4. The muscular coat in the form of an inner circular and an outer longitudinal series of fibres, both of which are striated (Figs. 14 and 15).

5. The connective tissue coat. It is an extremely thin membrane which invests the outer surface of the muscle fibres, and also lines the quadrangular spaces formed between them. In the latter case this coat is in contact with the basement membrane.

The epithelium lining the anterior third of the stomach is very uniform in character (Fig. 25). Towards its middle, however, they are seen to alter and give off protrusions into its cavity (Fig. 15). These protrusions are finely granular, and nearly transparent; they stain only extremely faintly. They are composed of accumulations of secretion which eventually become detached as spherical masses. In these cells the chromatin granules were observed to have a tendency to become concentrated into the centres of the nuclei. Van Gehuchten has specially studied the digestive system of the larva of *Ptychoptera contaminata*, and has described similar cells. He explains their function as follows. When secretion commences within the cells the clear fluid elaborated in their protoplasm increases the intra-cellular tension until, eventually, the fluid breaks through certain weak places in the mem-



brane of the cell and bulges out into the lumen of the mid-gut in the form of a pear-shaped vesicle of a liquid rich in albumens, at first attached to the free face of the cell, but eventually becoming set free (1890, p. 238). In *Anopheles* these protrusions can be seen along the epithelium of the stomach nearly to where it joins that of the intestine.

Distributed at irregular intervals at the bases of the epithelial cells are small pear-shaped cells (Fig. 15). They lie between the larger cells and are disposed either singly or in groups of two or three. Similar cells to these are common in the mid-gut of various insects. It is probable that after the epithelial cells have burst and discharged their secretion, these small cells are the centres from which the epithelium is regenerated. These cells are extremely well shown in the stomach of the cockroach (*Periplaneta*), and Miall and Denny have pointed out their close resemblance to Watney's buds in Mammals. In this insect they have described all the stages in the development of new epithelium from these buds that have been noted by Watney in the case of Mammals.

The epithelial cells of the whole mesenteron occasionally exhibit a well-marked "striated margin" or "Härenchensaum" along their free edge bordering the gut cavity. I have found it to be best and most frequently seen in the cells of the caecal diverticula; the only place where I have not observed it with any certainty is on the large columnar cells of the cardia, but I do not wish to infer that those cells never develop this structure. In those preparations where orange G has been used as a second stain, the striated border shows up very distinctly, and appears rich yellow in colour (Fig. 18). The presence of this border is eminently characteristic of cells in the resting condition. It is very often to be seen in preparations of the mid-gut of various insects, and it bears a close resemblance to the "striated hem" found in the intestinal epithelium of Vertebrata. A great deal of diversity of opinion exists as to its structure, origin, and function. Under a high magnification it *appears* to be made up of extremely delicate and closely-set vertical processes or filaments, which rest on a basal membrane. The best and most recent account of this structure is given by Vignon (1901, p. 371).

The peritrophic membrane may be regarded as a thin tube which completely encloses the food as it passes through the mesenteron. It is quite colourless and, on account of its resistance to the action of alkalies, it is inferred to be of a chitinous nature. It is only to be



seen, as a rule, in actual contact with the mesenteric epithelium at its point of origin near to where the mid-gut joins the oesophagus. Over the rest of the gut it is separated from the epithelium by a space of variable width, and usually filled with a granular fluid most probably of a secretory nature. The membrane crosses the apertures of the caeca, but is not reflected into their cavities (Fig. 25), and it extends for a considerable distance into the hind-gut (Figs. 20 and 25). An examination of the exuviae of *Anopheles* larvae shows that during the periods of ecdysis the peritrophic membrane is shed and got rid of through the anus. In some instances it was seen in a crumpled condition in the hind-gut prior to being evacuated. In others, the membrane was seen to be double, one tube lying within the other. This occurrence is to be explained by the fact that the old membrane was still *in situ*, and the new one had already been formed around it.

A chitinous lining to the mid-gut is of frequent occurrence in most orders of Insects, as well as occurring in the Myriapoda, certain Crustacea, and a few Gasteropoda (*Helix*, *Limax*, and *Lymnaea*) (Schneider, 1890, p. 92). How far the membrane is strictly homologous in these various groups it seems at present impossible to say. With regard to its origin among Insecta, very diverse views are held and, moreover, it is extremely probable that its mode of development differs among various insects. Gehuchten (1890, p. 273) has studied it in the larva of *Ptychoptera*, Cuénot (1895, p. 293) in the Orthoptera, Vignon (1901, pp. 382, 396, and 537) in the larva of *Chironomus* and in the "silkworm," and Balbiani (1890, p. 1) and Plateau (1878, p. 85) in the Myriapoda. All these observers agree in regarding it as being a product of the cells of the mesenteron. Balbiani, Plateau and Vignon (in the silkworm) state that it is a secretion formed at the surface of the whole of the epithelial lining of the mesenteron. Gehuchten, Cuénot and Vignon (in the *Chironomus* larva) regard it as being secreted in a localised area of the cardia. Schneider (1890, p. 89) has studied this membrane very briefly in a number of Insecta, and terms it the funnel ("Trichter"). He states that it is a backward continuation of the cuticular lining of the oesophagus, but he did not study its process of formation. If his observations be substantiated by future enquiry, it would seem that there are two types of this membrane to be found among the Insecta which are analogous, and not homologous with one another. In the one case it would seem that it is a product of the ectoderm (Schneider), and in the other a product of the endoderm (the other authorities quoted). However it may be for some of the forms Schneider has studied, it is far from certain whether it is of the nature he claims it to be in the case of the *Chironomus* larva. The detailed researches of Vignon go a long way towards proving that it really arises as a chitinous secretion, at first of a fluid nature, but becoming coagulated a short time after its emission, and produced by a row of thickened epithelial cells situated in the anterior region of the cardia.

After a study of a number of prepared sections of the mid-gut of the *Anopheles* larva, I am inclined to believe, with Vignon and others, that the peritrophic membrane arises in the anterior part of the cardia. The large deeply-staining columnar cells which are characteristic of this region, and have already been referred to (p. 303), have all the characters of very active secretory cells, and I regard them as being the seat of origin of this membrane. The latter commences close to the point where these cells arise, and in some instances I have observed it in close contact with the inner face of this epithelium (Fig. 24); elsewhere it only *touches* the walls of the gut in places. It seems most likely that the membrane, as it is being secreted, is pushed backwards into the stomach by the food as it (the food) issues through the oesophageal valve.

With regard to the function of the peritrophic membrane, the most probable suggestion is that it seems to protect the mesenteric epithelium from abrasion by hard and resisting particles of food.

Schneider states that a chitinous membrane is present in the mid-gut of Thysanura, Orthoptera, in many Neuroptera and Coleoptera, in ants and wasps among Hymenoptera, and in larvae of Diptera and Lepidoptera. He mentions that it is wanting in *Carabus*, *Dytiscus*, *Coccinella* and *Bruchus* among Coleoptera, in some Hymenoptera, in the adults of Lepidoptera and in Hemiptera. He claims that all those insects (together with their larvae) which possess the membrane or "funnel," as he terms it, eat solid and indigestible substances for food, while those which do not possess it take their food in a fluid form.

Since the food is separated off from the mesenteric epithelium by means of this membrane, the question arises as to how the digestive secretions come into contact with the food and the method by which absorption takes place. Gehuchten (1890, p. 272) states that "sans aucun doute" the digestive secretions traverse the peritrophic membrane by means of osmosis, and by a similar means the elaborated products come in contact with the epithelium ready for absorption. Vignon (1901, p. 538) explains the process in a similar manner. Miall and Hammond (1900, p. 58) remark that a granular fluid is present in the narrow space between the epithelium and the membrane; it also contains granules of larger size which they believe to come from the food. They believe, however, that it is unnecessary to suppose that the secreted fluid *diffuses through* the peritrophic membrane; the granules just noted indicate that another communication exists. They believe it probable that the fluid squeezed out from the food in the oesophagus and oesophageal valve passes down the cylindrical tube

formed by the peritrophic membrane, and that it is regurgitated into the outer space by the contractions of the powerful circular muscles situated at the commencement of the small intestine. Furthermore, Dell (1905, p. 293) states that he has observed reversed peristaltic contractions in the intestines of the living *Psychoda* larva. The digested food has been seen to be carried up into the space between the peritrophic membrane and the mesenteric epithelium by this means. In the case of the larva of *Anopheles* and other insects, however, the peritrophic membrane does not cease at the termination of the mid-gut, but is prolonged for some distance into the hind intestine. In such instances regurgitation would of necessity take place very far backwards, and if this took place the products of digestion would surely become liable to be contaminated with faecal matter. In other cases, the peritrophic membrane extends as far backwards as the anus, and under such conditions regurgitation could not take place at all.

The theory of diffusion seems to explain the process more adequately and does not present any very great difficulties. It is well known that animal membranes are permeable to salts and sugars but not to proteids, which are of the nature of colloids (non-diffusible substances). The proteids of lower molecular weight, namely the proteoses and peptones, however, are highly diffusible when compared with albumin (Schäfer, 1898, p. 45). The action of the digestive secretion would, however, most likely convert the albumins of the food into substances analogous to peptones, and in this way the difficulty would be obviated.

*The Hind-gut:*—The Hind-gut commences about the middle of the sixth abdominal segment (Text-Fig. p. 298). It may be divided into three regions, viz. *ileum*, *colon* and *rectum*.

The *Ileum* receives at its commencement five Malpighian tubes (*M.t.*), and extends to the beginning of the eighth segment of the abdomen. It is lined by a flattened epithelial layer, but it was not possible to distinguish any cell boundaries; an extremely delicate intima invests the cells where they border the cavity of the gut (Fig. 17). The epithelium rests on a basement membrane (*b.m.* in Fig. 17), and situated outside the latter is a very strongly developed coat of circularly disposed muscle fibres, and they are succeeded by a layer of longitudinal muscles. The whole is covered exteriorly by a thin connective tissue membrane. The circular muscles are extremely closely packed together, each band being in contact with its fellows. A short distance beyond the commencement of the gut they attain a great thickness (Fig. 17).

The *Malpighian tubes*, as already stated, are five in number, the odd tube being situated on the dorsal side (Fig. 3). This same number is present in the adult mosquito. They have the usual structure seen in insects. From the point where they arise from the gut, up to about where they form a loop to redouble and pass backwards, the limits between the individual cells of their epithelial lining are scarcely indicated, but over the rest of their course the cells are very distinct and bulge somewhat into the central cavities of the tubes (Figs. 3 and 21).

The protoplasm of the cells contains an abundance of dark granules, most probably of an excretory nature. Externally, the tubes are invested by a membrane of connective tissue. Five is a very unusual number for the Malpighian tubes, nevertheless it is present also in the larvae of *Culex*, *Psychoda*, *Ptychoptera*, and of the Blepharoceridae. For this reason Müller (1881, p. 499) has proposed to unite these forms together into a common group which he terms the "Pentanephria." According to Eysell (1902, p. 341) five Malpighian tubes apparently also occur in *Aedes cinereus* (imago).

The *Colon* is a much wider tube than the ileum, and it lies mostly within the eighth abdominal segment (Text-Fig. p. 298); it passes by a gradual transition into the rectum. Its epithelial lining is well developed, and consists of large polygonal cells with prominent nuclei, though the cell boundaries are not always to be distinguished. It is lined internally by a thin intima (Figs. 19 and 20). The colon is provided with a strongly-marked coat of circular muscles (Figs. 3, 19 and 20); these bands are placed at very regular intervals from one another. When the alimentary canal of the larva is dissected out these muscles show very prominently on the exterior (Fig. 3), but they do not, however, attain the thickness of those of the ileum. Externally, the colon is invested by connective tissue.

The terminal chamber of the hind-gut or *Rectum* (Fig. 3 and Text-Fig. p. 298) can be distinguished from the colon by its very much thinner epithelial lining, which resembles that of the ileum. It is lined by a delicate cuticle or intima, and its circular muscles are disposed in a similar fashion to those of the colon, only they are not so pronounced when viewed in section. There are no longitudinal muscles.



*The Respiratory Organs.*

The Respiratory Organs of the *Anopheles* larva consist of the tracheal system, and possibly the two pairs of anal processes situated around the hinder extremity of the body are of the nature of gills.

The *Tracheal System* (Fig. 27) communicates with the atmosphere by means of a single pair of spiracles located on the dorsal aspect of the eighth abdominal segment. It is, therefore, an example of the metapneustic arrangement.

There are two principal longitudinal trunks, lying one on either side of the mid-dorsal line of the body (*l.t.*). These vessels run from the spiracles directly forward into the thorax. In the latter region each trunk divides into a pair of branches which supply the various organs of the head. From the two main longitudinal trunks smaller tracheae arise which pass to the different organs in the body, and to a very large extent they maintain a segmental arrangement. In each abdominal segment (excepting the ninth) an outwardly directed vessel is given off on either side (*t.t.* in Figs. 27 and 28). It soon bifurcates into an anterior and a posterior branch; the anterior branch anastomoses with the posterior one of the segment in front, and the posterior branch with the anterior one of the segment behind. In this fashion a secondary longitudinal trunk is formed along each side of the body. Where this latter vessel meets the transverse vessel (*t.t.*) in each segment, a branch arises which chiefly supplies the gut and part of the musculature (*o.* in Fig. 28). A short distance behind it there is similarly repeated in each segment a second branch (*p.*) which, passing downwards, divides into numerous twigs supplying the nerve cord and ventral muscles. At the point of its bifurcation each transverse vessel is continued outwards to the integument in the form of a delicate strand (*stig.c.* in Fig. 28). The point where the latter meets the external cuticle is marked by a minute chitinous scar. These strands, or stigmatic cords as they are sometimes termed, are perhaps to be regarded as the vestiges of the primitive invaginations which form the initial tubes leading from the spiracles to each segmental system in holopneustic insects. In the case of *Anopheles*, however, since all the spiracles, with the exception of the posterior pair, have been closed up, the initial tubes, having no further use, have atrophied into chitinous cords or strands. This seems to be brought about through their lumina becoming obliterated by means of a chitinous deposit. The stigmatic cords in the present larva are



difficult to make out on account of their small calibre and their transparency. They are to be seen in many Dipterous larvae, and can be studied with greater facility in the larva of *Eristalis*, among others, than in that of *Anopheles*.

The two longitudinal trunks are united with one another by means of segmentally repeated commissures. In the region of the eighth abdominal segment the two main trunks give off a great number of small branches along their inner and ventral aspects (Figs. 27, 30 and 31). It has not been possible to detect any traces of the spiral thickening in these branches, and I believe that they are without it. At their origins from the main trunk these branches have a tendency to be united together into small bundles opening into depressions or crypts in the walls of the former (Figs. 31 and 33). Traced further along their course they are seen to separate apart, and to pass to the walls of the terminal chamber of the heart (Fig. 30). On account of the thinness of the walls of the ultimate branches (or capillaries) of these tracheae, it seems very probable that the blood is brought into close contact with the oxygen contained in them, and in this way a kind of "lung" is formed.

In addition to *Anopheles* I find, after an examination of the larvae of *Dixa* and *Culex*, both by means of dissections and serial sections, that a similar arrangement is present in them. In the case of *Dixa*, the branches were seen to arise almost immediately beneath the spiracular apertures, and some of them went directly to the heart, while others appeared to float freely in the blood space contained within the segment. In *Culex* the arrangement is identical with that of *Anopheles* (Fig. 29), excepting that the branches are not quite so abundant. Raschke (1887, p. 133), however, in his description of the larva of *Culex nemorosus*, makes no mention of this feature, but he states that in association with the anterior end of the rectum are great numbers of fine tracheae which are devoid of the spiral thickening. He states that they pass through the wall of this portion of the gut and, subdividing, terminate in countless numbers of minute twigs lying in papilla-like folds situated within the rectum. This arrangement of the tracheae is not represented in any of his figures of the larva, and I have been quite unable to discover any traces of it after having examined a considerable number of *Culex* larvae. Furthermore, Vaney (1902, p. 138) in his studies of Dipterous larvae, mentions the relationship just described that exists between the tracheal system and the heart, but makes no reference to the presence of tracheae in the walls

of the rectum. Thompson (1905, p. 145) in his detailed account of the alimentary canal of *Culex* likewise makes no mention of it. In the light of this evidence I would suggest the probability that the branches described by Raschke are the identical ones which are seen in Fig. 29, and, therefore, is it not probable that as these branches lie immediately over the rectum, and obscure most of the heart from view at the point where they are situated, Raschke has been deceived into believing that they supply the rectum?

A similar disposition of the tracheae in relation with the heart is mentioned by Vaney (1902, p. 138, and Plate IV, Fig. 59) in the larva of *Psychoda sexpunctata*, and he figures the two bunches of tracheae arising from the main longitudinal trunks. Dell (1905, p. 300) in his paper on this larva says that "In the last segment, immediately dorsal to the hinder part of the heart, there arise from each longitudinal trachea a number of small branches, which break up into branches of distribution in the neighbourhood of the pericardium." Neither of these observers, however, have devoted more than a passing reference to this relationship of the tracheae.

A somewhat similar system, by means of which the haemocoelic and tracheal systems are brought into close relationship with one another, is described by Viallanes (1882, p. 65) in the case of a larva living in mud, and which he believes to belong to the genus *Ctenophora*. In this instance he states that the dorsal vessel is in the form of a tube open at its anterior and posterior extremities, and there are no valves or ostia. He believes that this condition coincides with a peculiar arrangement of the tracheal system. The larva in question possesses a pair of spiracles situated at the hinder extremity of the body. From either spiracle there runs throughout the length of the animal a main tracheal trunk, and just in front of the middle of the last segment a transverse connection joins the two trunks together. It is at the level of this anastomosis that the dorsal vessel commences. Between the spiracles and this anastomosis the two main tracheal trunks give off in all directions a great number of small branches. These branches float freely in the cavity of the last segment, where they are bathed with the blood it contains, and they are present in such numbers as to occupy the greater part of it. In this instance the blood receives oxygen from the tracheae before entering the heart.

The above cases are the only instances that I have been able to come across where the tracheal and circulatory systems are in intimate connection with one another. The question arises as to why this arrangement should occur in the particular forms mentioned, and apparently be wanting in most other Dipterous larvae. As a possible solution, I would suggest that perhaps it is correlated with a reduction in the number of the spiracles. It will be observed that the larvae of *Anopheles*, *Culex*, and *Dixa* are all metapneustic forms, and similarly the larva regarded by Viallanes as being that of a species of *Ctenophora*.

In the case of the *Psychoda* larva, in addition to the posterior spiracles, there is also a minute pair situated on the first (or prothoracic) segment. In all probability this latter pair of spiracles are examples of vestigial organs, for indeed Dell (1905, p. 299) remarks that "they are not open and probably not functional, since they are always immersed in water or mud." If this be true, although the larva is morphologically amphipneustic, physiologically it is metapneustic, as in the other instances quoted. It would be rash, in the light of these few instances, to regard this condition as being characteristic of metapneustic larvae, and nothing more than the possibility of its occurrence being correlated with that type of tracheal system is here suggested. Just as Prof. Miall (1891, p. 458) has remarked with regard to the distribution of haemoglobin, that we have a tolerably satisfactory reason for its occurrence in a number of aquatic animals whose respiration is limited, and whose surroundings make it a matter of difficulty to procure a sufficient supply of oxygen, we have, however, to admit that many similar animals, under the same conditions, manage perfectly well without it. In the same way, very probably, many metapneustic Dipterous larvae will be found where this relationship between the tracheal system and the heart is likewise dispensed with. Such an admission is not a logical refutation of the explanation. In those larvae, where there is but a single pair of functional spiracles, it is by no means unreasonable to believe that the passage of oxygen to the various organs of the body must be an extremely slow process. There is the possibility of this disadvantage being eliminated in the instances related above, for the blood as it is pumped forwards would absorb oxygen directly from the tracheae, and in this way the oxygenation of the tissues would be greatly expedited. I am fully aware, however, how difficult it is to give a correct interpretation of natural contrivances, and how easy it is to formulate an idea as to how a certain fact may be explained. As Semper (1899) remarks, there is very little trouble needed to imagine some process by which hypothetical fundamental causes may have led to the result which has been actually observed.

The spiracles are situated close to the middle line on the dorsal aspect of the eighth abdominal segment (Fig. 27). They are supported by a complex chitinous skeleton, which is a development of the tergum of that segment; it has been described in detail by Nuttall and Shipley (1901, p. 64). The spiracles lie fully exposed to the air, and are of an extremely simple structure, being little more than simple chitinous

rings which lead directly into the main tracheal trunks. Almost immediately within their apertures the walls of the tracheae are seen to be covered with a layer of branched chitinous trabeculae (Figs. 31 and 32). These trabeculae extend along the ventral walls to the point where the tracheal branches are seen to take their origin; dorsally, however, they extend some distance further inwards (Fig. 31).

The *Anal* (tracheal) *Gills* consist of two pairs of delicate leaf-like outgrowths of the integument situated around the vent. The two gills of each pair are placed dorso- and ventro-laterally respectively in relation to that aperture. The cavities of the gills are in free communication with the general haemocoelic cavity of the animal (Text-Fig. p. 298) and, therefore, contain blood, and they are capable of a considerable amount of extension and retraction, which depend upon the quantity of blood contained within them at a given time. Each pair of gills is supplied by a tracheal branch arising from the main air-trunk close to the spiracle of its side (*g.t.* in Fig. 27). Just before reaching the gills it bifurcates into two branches, one passing to each gill. Within the gill the branch immediately sub-divides, and the capillaries thus formed do not undergo further sub-division, but pass straight to the apex of the organ. Here they redouble and pass backwards for some distance.

On account of the thinness of the integument investing these organs, and the fact that they are well supplied with blood and tracheae, there is good reason to believe that they function as accessory respiratory organs. They are very characteristic of Culicid larvae, but are not to be confounded with the anal gills of the Chironomidae which contain blood but no tracheae. It is noteworthy that, according to Christophers (1906), in the larva of a *Stegomyia* (*scutellaris*?) they are extremely large and, moreover, the latter has a habit of remaining for long periods at the bottom of the water.

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## REFERENCE LETTERING.

***	(Fig. 31) Direction of the section in Fig. 32.
A, A.	Palmate hairs.
abd <sub>8</sub> .	Eighth abdominal segment.
abd <sub>9</sub> .	Ninth ditto.
a.g.	Anal gills.
an.	Anus.
ant.	Antenna.
ap.	Aperture of salivary duct.
B.	Stigmatic plate.
b.	Brush.
b.m.	Basement membrane.
br.	Tracheal branches passing to heart.
b'r'.	Ditto in transverse section.
b.s.	Blood space.
b.sin.	"Blood sinus."
c.	Cuticle.
car.	Cardia.
c.e.	External layer of the cuticle.
c.i.	Internal       ,,       ,,
c.p.	Chitinous plate.
c.m.	Circular muscles.
c'.m'.	Connective tissue membrane.
co.	Colon.
coe.	Caeca.
crp.	Tracheal crypt.
e.	Epipharynx.
ep.	Epithelium.
ex.w.	External surface of cardia.
fr.h.	Frontal hairs.
f.g.	Frontal ganglion.
g.t.	Tracheal trunk supplying anal gills.
h.	Hypodermis.
hs.	"Härchensaum."
ht.	Heart.
il.	Ileum.
in.	Intima.
i.w.	Inner wall of oesophageal valve.
l.	Labrum.
lb.	Labial plate.
lig.	Ligament of connective tissue.
l.m.	Longitudinal muscles.
lt.	Principal (or lateral) tracheal trunk.
l'.t'.	Secondary lateral tracheal trunk.
m.	Mandible.

<i>m'</i> .	Greatly thickened circular muscles of oesophagus.
<i>m<sub>1</sub></i> .	Epipharyngeal muscles.
<i>m<sub>2</sub></i> .	Anterior lateral pharyngeal muscles.
<i>m<sub>3</sub></i> .	Lateral pharyngeal muscles.
<i>m<sub>4</sub></i> .	Dorsal pharyngeal muscles.
<i>m<sub>5</sub></i> .	Longitudinal pharyngeal muscles.
<i>m<sub>6</sub></i> .	Elevator muscles.
<i>m<sub>7</sub></i> .	Lateral dilator muscles.
<i>m<sub>8</sub></i> .	Diagonal muscles.
<i>m<sub>9</sub></i> .	Retractor pharyngeal muscles.
<i>ms</i> .	Mesenteron.
<i>mx</i> .	Maxilla.
<i>mx.p.</i>	Maxillary palp.
<i>n</i> .	Nucleus.
<i>n'</i> .	Nerve fibre (?).
<i>nc</i> .	Nucleolus.
<i>o</i> .	Tracheal branch to gut and muscles.
<i>oes</i> .	Oesophagus.
<i>o.w.</i>	Outer wall of oesophageal valve.
<i>p</i> .	Tracheal branch to nerve cord and ventral muscles.
<i>ph</i> .	Pharynx.
<i>p.m.</i>	Peritrophic membrane.
<i>prc</i> .	Dorsal notched process.
<i>r</i> .	Rectum.
<i>rd</i> .	Chitinous rod-like thickening.
<i>s</i> .	Groups of setae.
<i>s.c.</i>	Stigmatic cord.
<i>s.d.</i>	Salivary duct.
<i>sec</i> .	Fluid contents of caeca.
<i>s.g.</i>	Sub-oesophageal ganglion.
<i>s.gl.</i>	Salivary gland.
<i>si</i> .	Respiratory siphon.
<i>sk</i> .	Supporting skeleton (stigmatic plate) of spiracles.
<i>s.o.</i>	Probable sense organs.
<i>sp</i> .	Spiracle.
<i>sub.hd.</i>	Sub-hypodermal tissue.
<i>t</i> .	Tooth-like projections.
<i>t.p.</i>	Tergal plate.
<i>tr</i> .	Trachea.
<i>trab.</i>	Chitinous trabeculae.
<i>t.t.</i>	Transverse tracheal trunk.
<i>x</i> .	Bulging in inner wall of oesophageal valve caused by food in stomach.

## EXPLANATION OF PLATES IV and V.

## PLATE IV (Figs. 1—20).

Fig. 1. Fully grown larva, dorsal aspect  $\times 16$ . [From Nuttall and Shipley.]

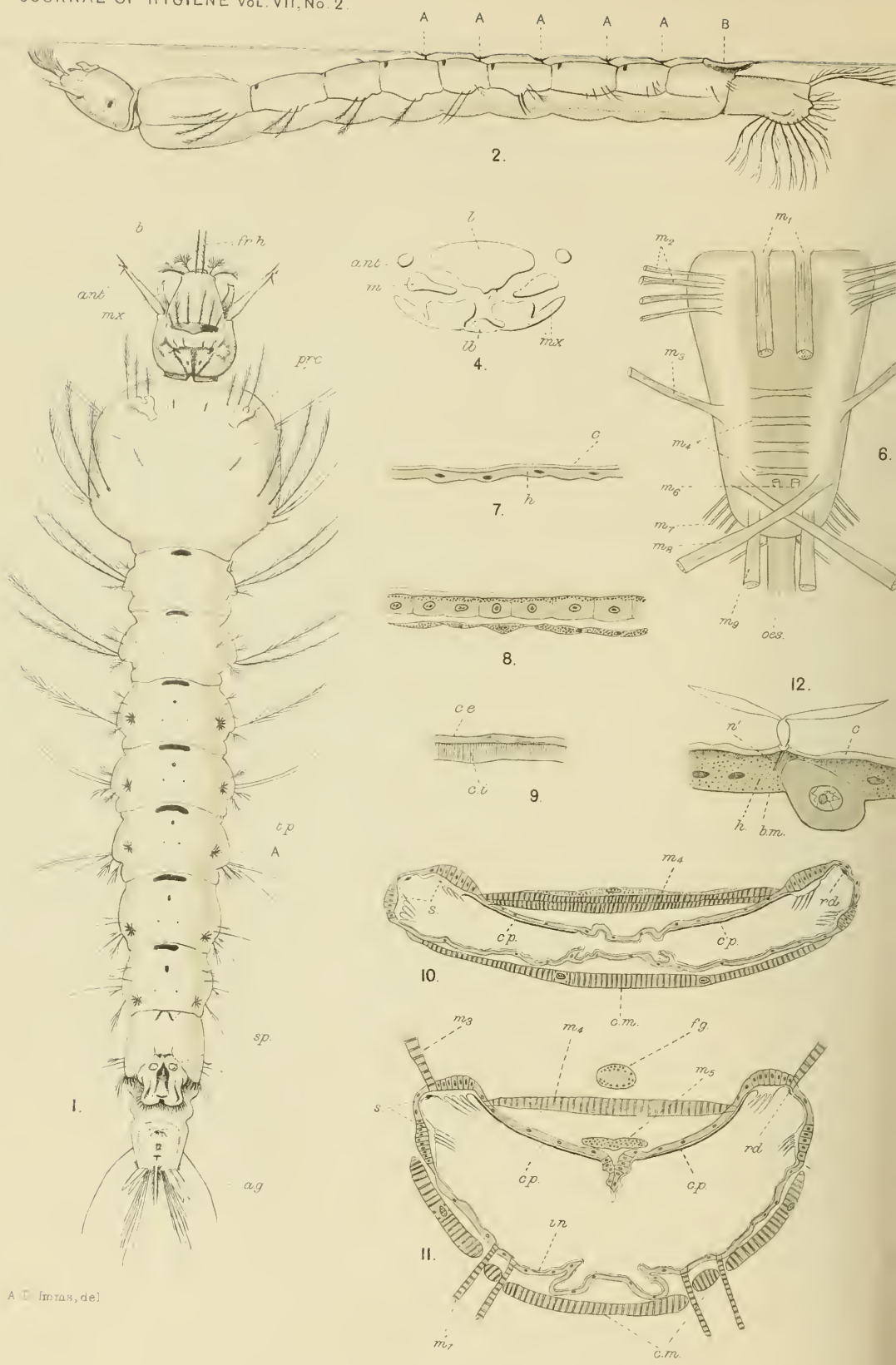
Fig. 2. Fully grown larva viewed laterally to show method of attachment to surface film of the water  $\times 12$ .

- Fig. 3. The digestive canal of a fully grown larva.  
 Fig. 4. Diagram to show the relations of the mouth-parts to one another.  
 Fig. 5. Longitudinal and slightly oblique section taken through the mouth and pharynx together with the salivary duct (very young larva).  
 Fig. 6. Dorsal aspect of the pharynx showing its musculature; reconstruction from serial sections. (Diagrammatic.)  
 Fig. 7. Section through the integument.  
 Fig. 8. Section through the integument from the median dorsal region of the abdomen shewing sub-hypodermal tissue.  
 Fig. 9. Section through the cuticle.  
 Fig. 10. Transverse section through the commencement of the pharynx.  
 Fig. 11. Transverse section of the pharynx, taken a short distance beyond the middle.  
 Fig. 12. Section through palmate hair and trichogenous cell at its base.  
 Fig. 13. Section through the bases of two feathered hairs of the abdomen together with their trichogenous cells.  
 Fig. 14. Section through the wall of the stomach.  
 Fig. 15. Ditto, showing protrusions.  
 Fig. 16. Transverse section of the oesophagus near its commencement.  
 Fig. 17. Transverse section through the commencement of the ileum.  
 Fig. 18. Portion of the wall of one of the caeca of the mesenteron; the cells show a marked "härchensaum" along their inner faces.  
 Fig. 19. Transverse section through the colon.  
 Fig. 20. Longitudinal section through the colon.

PLATE V (Figs. 21—33).

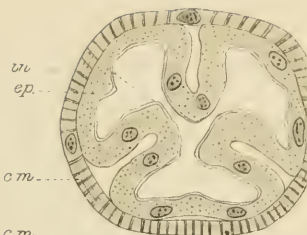
- Fig. 21. Longitudinal section taken through the junction of the mid-gut and hind-guts and passing through the commencement of one of the Malpighian tubes.  
 Fig. 22. Transverse section across a salivary gland at its widest part.  
 Fig. 23. Nucleus of secretory cell of salivary gland.  
 Fig. 24. Section through the glandular part of the epithelium of the cardia showing the peritrophic membrane in close contact with the inner surface of the cells.  
 Fig. 25. Longitudinal section through the oesophageal valve, the cardia and two of the caeca.  
 Fig. 26. Transverse section through the line *a...b* in the preceding figure: partly diagrammatic.  
 Fig. 27. General view of the tracheal system.  
 Fig. 28. Figure showing the segmental tracheal systems in two of the abdominal segments: the arrow is pointing towards the head of the larva.  
 Fig. 29. Hinder portion of the abdomen of a *Culex* larva showing numerous branches, passing from the two main tracheal trunks to the heart.  
 Fig. 30. Horizontal and longitudinal section through the posterior extremity of the heart showing numerous tracheal branches passing to its walls.  
 Fig. 31. Longitudinal section through the left spiracle and the commencement of the main trachea.  
 Fig. 32. Transverse section through the line *\*...\** of Fig. 31, showing the trabeculae arising from the walls of the trachea.  
 Fig. 33. View of the inner surface of one of the main tracheal trunks showing the "crypts," and the numerous apertures of the branches which pass to the heart.



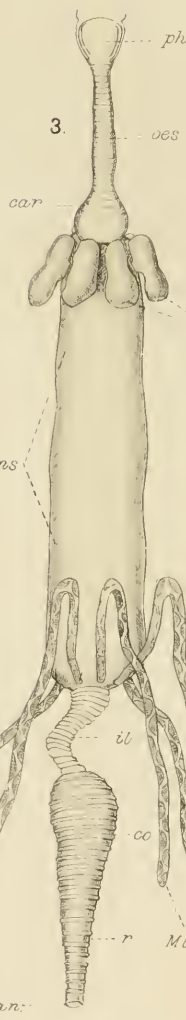




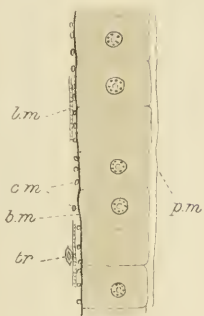
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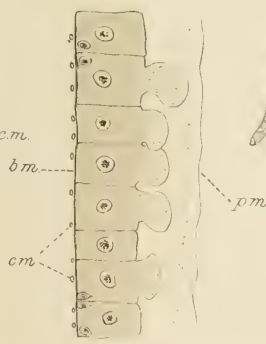
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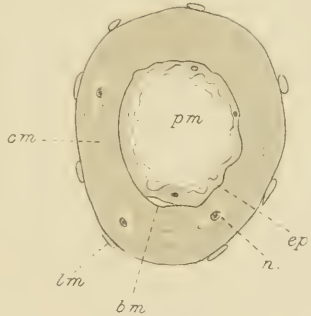
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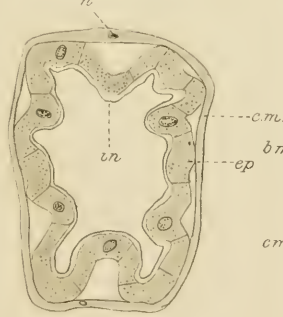
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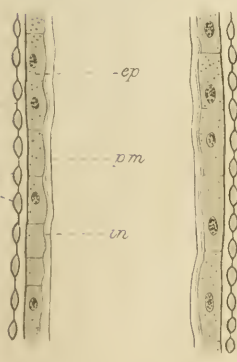
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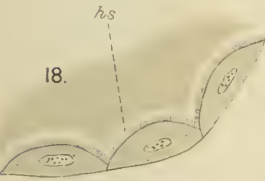
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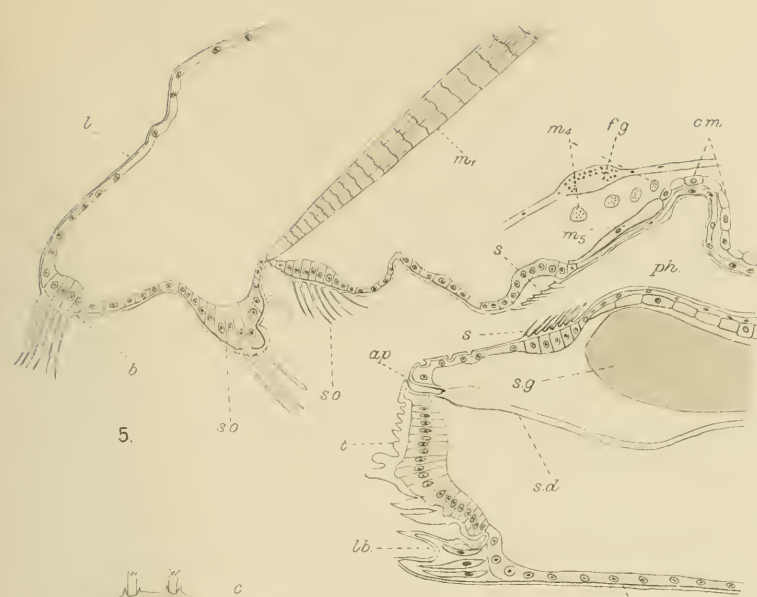
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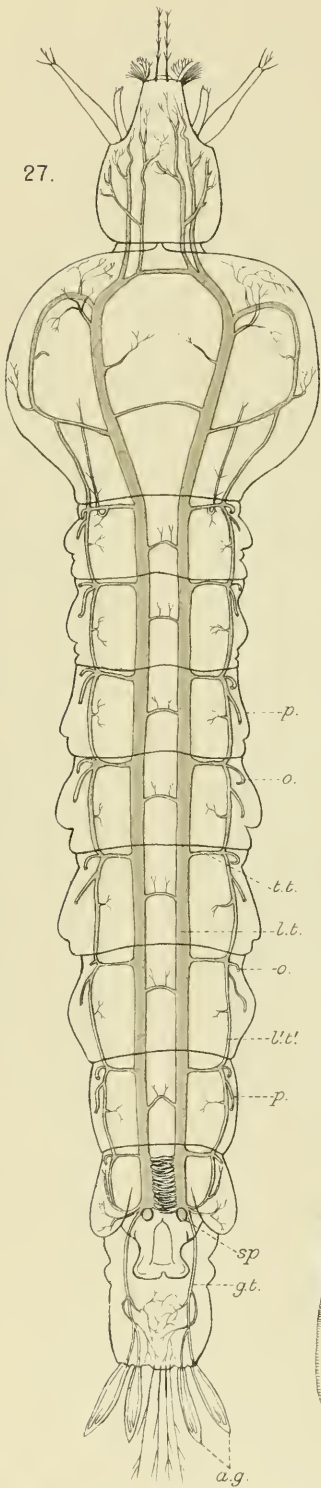


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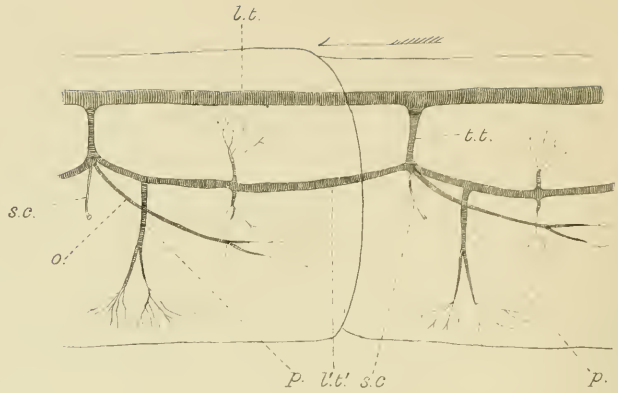




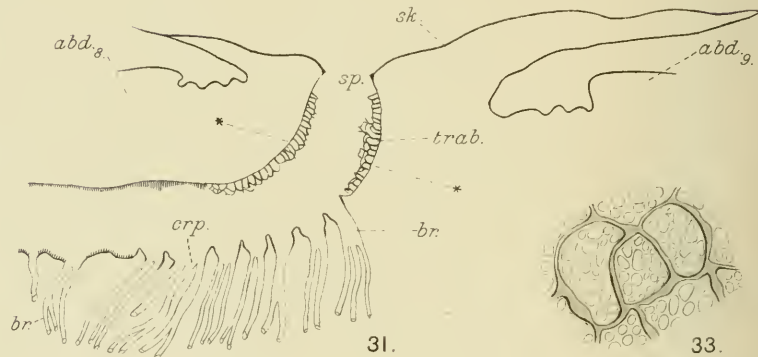




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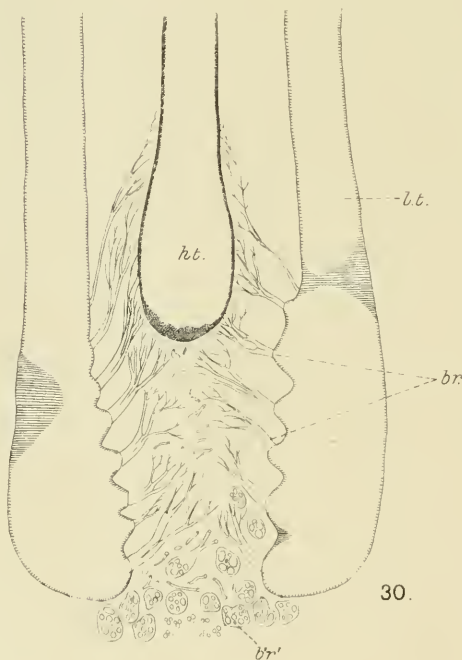
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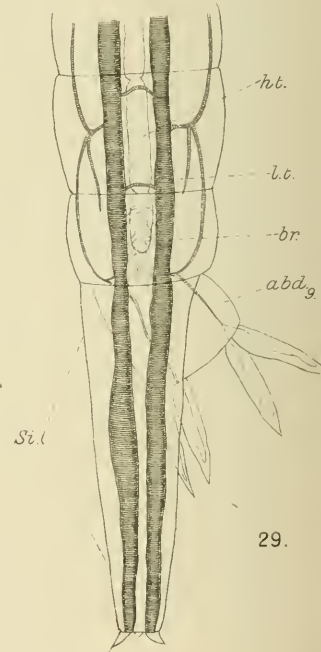
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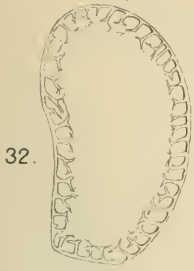


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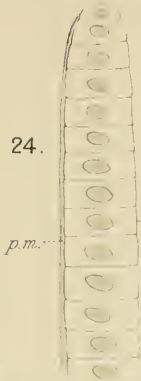


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24.



p.m.

ea.w.

car.w.



oes.

m.

lig.

25.

b.s.in

l.m.

o.w.

b.s.

oes.

x.

int.

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23.



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m.s.

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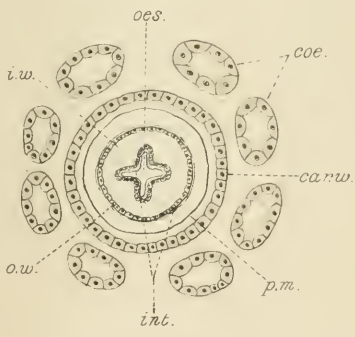
h.g.

l.m.

c.m.



26.



oes.

i.w.

coe.

car.w.

p.m.

int.

o.w.









*Photo: Elliott and Fry.*

**ALLAN MACFADYEN.**

Born 26 May, 1860, in Glasgow.

Died 1 March, 1907, in London.

## IN MEMORIAM. ALLAN MACFADYEN.

THE regretted death of Dr Allan Macfadyen on March 1st, at the early age of 46, removes a name well known and justly honoured from the all too small band of British Bacteriologists. Born in Glasgow, he received his medical and scientific education at the University of Edinburgh, of which he was a distinguished student, gaining the gold medal at the M.D. examination in 1886 and taking the B.Sc. degree two years later. He subsequently studied in Germany at Berne, Göttingen and Munich, under such masters as Carl Flügge, Nencki and Pettenkoffer, gaining an intimate knowledge of chemical and bacteriological methods and a mastery of the German language. From 1889 to 1892 he was a Grocers' Company Research Scholar, and about this time became Professor of Bacteriology at the old College of State Medicine, subsequently amalgamated with the British Institute of Preventive Medicine. At the Institute he succeeded Dr Ruffer as Secretary and had a large share in the planning and organisation of the new building at Chelsea. Here, at the Jenner and Lister Institute, as it successively became, he acted as Secretary to the Governing Body and was head of the Bacteriological Department, and much of the work which emanated from there was inspired by him. He had been Examiner in Hygiene at the University of Edinburgh, and was Fullerian Professor of Physiology at the Royal Institution 1901-1904.

One of Dr Allan Macfadyen's earliest investigations was on the behaviour of bacteria in the digestive tract, in which he proved that in the gastric juice and intestinal secretion we have but little protection against the organisms that find their way into the alimentary canal. This was followed by a joint paper with Nencki and Sieber on the chemical processes in the small intestine of man, in which advantage was taken of a patient with faecal fistula, to make analyses of the intestinal contents under different conditions of diet and to study the share taken by the numerous bacteria present in the decomposition of food. He also

studied the action of bacteria on protein bodies, and, with Sir Lauder Brunton, the ferment action of bacteria, showing that peptonising and diastatic enzymes may be produced by micro-organisms, and this work was continued in a paper on the biology of the ringworm organism published in 1895. The thermophilic and photogenic bacteria also attracted his attention and important contributions to the biology of these organisms were published with Dr Blaxall and Mr Barnard respectively. About 1899 the researches of E. Buchner on the alcoholic ferment of yeast greatly interested Dr Macfadyen and probably had a good deal to do with directing his ideas into the lines to which during the last years of his life he devoted himself. With Mr Rowland and the late Dr Morris a paper was published on Buchner's zymase, and an apparatus for grinding yeast cells was devised which formed the starting point from which the machine for disintegrating bacterial cells in the presence of liquid air was ultimately evolved. By the year 1900 or thereabouts, the idea that antitoxic and anti-microbic sera would prove a panacea for all infective diseases had been proved to be fallacious, and an *impasse* had been reached owing to the difficulty of obtaining anti-sera for the endotoxins or intracellular poisons of bacteria. Macfadyen conceived that if bacteria could be disintegrated and their juices obtained in such a manner that chemical changes were inhibited it might be possible to obtain toxins which on injection would stimulate the formation of the proper anti-bodies. By a series of researches it was successively shown that the virulence of an organism varied directly with the amount of endotoxin that could be obtained from it; that an animal might be immunised by means of these endotoxins, and that the serum of such an animal possessed immunising and curative properties. The application of these principles to the typhoid bacillus, cholera vibrio, pneumococcus, and hog-cholera bacillus was described in a series of papers, and, latterly, the treatment of disease in man by means of anti-endotoxic sera had yielded encouraging prospects. Somewhat prior to this work the employment of liquid air in the various researches naturally directed Dr Macfadyen's attention to the influence of low temperatures on bacterial life, and in a series of papers it was shown that little or no deleterious action on vitality or on biological properties was suffered by bacteria exposed to liquid air and even to liquid hydrogen. A man of kindly nature and genial disposition, Dr Macfadyen's death, a result of accidental infection with typhoid and Malta fever, will leave a blank not easily filled.

R. T. H.



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# REPORTS ON PLAGUE INVESTIGATIONS IN INDIA.

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA, THE  
ROYAL SOCIETY, AND THE LISTER INSTITUTE.

(Plates VII to XII.)

*(Continued from Volume VI, p. 536.)*

	PAGE
XI. The diagnosis of natural rat plague (Plate VII) . . . . .	324
XII. The pathological histology of the spleen and liver in spontaneous rat-plague, with observations on the experimental infection. By J. C. G. Ledingham. (Plates VIII and IX) . . . . .	359
XIII. Transmission of plague by feeding rats with infected material . . .	373
XIV. The significance of the locality of the primary bubo in animals infected with plague in nature . . . . .	382
XV. Further observations on the transmission of plague by fleas, with special reference to the fate of the plague bacillus in the body of the rat flea ( <i>P. cheopis</i> ) . . . . .	395
XVI. Experimental production of plague epizootics among animals (2nd communication). . . . .	421
XVII. Experiments in plague houses in Bombay (2nd communication) . . .	436
XVIII. On the external anatomy of the Indian rat flea ( <i>P. cheopis</i> ), and its differentiation from some other common fleas (Plates X to XII) . . .	446
XIX. On the natural occurrence of chronic plague in rats. No. 2 . . . .	457
XX. A note on man as a host of the Indian rat flea ( <i>P. cheopis</i> ) . . . .	472

## XI. THE DIAGNOSIS OF NATURAL RAT PLAGUE.

- I. Introduction.
- II. Preliminary details.
- III. Characteristic appearances in plague rats recognisable by naked-eye examination.
  - (1) The presence of rigor mortis, subcutaneous congestion, subcutaneous haemorrhages, and subcutaneous oedema.
  - (2) Changes in the lymphatic glands—buboes.
  - (3) Appearances which may be observed in the abdominal organs.
  - (4) Appearances which may be observed in organs in the thorax.
- IV. The value of certain characteristic post-mortem features in the diagnosis of plague rats, including animals which have undergone putrefaction.
- V. Plague-like diseases in the rat.
- VI. Remarks on the results of microscopical examination.
- VII. On the relative value of the methods of the naked-eye and microscopical examination in the diagnosis of rats suspected of being plague-infected.
- VIII. On diagnosis by the cutaneous inoculation of guinea-pigs with remarks on the virulence of the bacilli in naturally infected rats.
- IX. Concluding remarks.

### I. INTRODUCTION.

In the following paper an attempt will be made to give a description of certain characteristic features which we have observed on post-mortem examination of a large number of plague rats in the course of the daily routine rat investigation in Bombay. An account of the diagnosis of rat plague may appear at first sight to cover well-known ground and to be consequently unnecessary. Having regard however to its importance the subject seems to have received remarkably little attention, judging from the scattered references to it in the literature.

At least three reasons appealed to us as rendering it imperative to make a thorough investigation of the post-mortem features of plague rats—firstly to estimate the value of naked-eye examination *per se* as an aid in the diagnosis of rats suspected of being plague-infected—secondly to determine to what extent macroscopical examination alone might be

relied upon for purposes of diagnosis in the daily routine examination of rats sent to the laboratory—and lastly to elicit whether examination of a large number of plague-infected rats might reveal evidence of the mode of infection in these animals in nature—a question of crucial importance from the point of view of the epidemiologist.

The records on which the following account is based have been grouped into two series, which will be referred to hereinafter as Series I and Series II respectively. Series I comprises 200 plague rats (100 *M. rattus* and 100 *M. decumanus*<sup>1</sup>) from those examined during the off season, *i.e.* from July to December 1905, when sporadic cases only were occurring in rats and in men. All these rats were in a fresh condition, *i.e.* they showed no obvious signs of putrefaction. Series II consists of 4000 rats from those obtained during the early period of the epizootic, *i.e.* from the beginning of January to the middle of February 1906.

## II. PRELIMINARY DETAILS.

The following preliminary details may be given of the rats in the two series.

TABLE I. *Series I.*

			100 <i>rattus</i>	100 <i>decumanus</i>	Percentage of total
Young males <sup>2</sup>	...	...	9	7	8
Adult males	...	...	51	54	52·5
Young females	...	...	8	9	8·5
Adult females	...	...	32	30	31
Pregnant	...	...	4	2	3
Rats brought alive for examination			5	6	5·5

TABLE II. *Series II.*

Analysis of 31,174 rats examined from 1-I-06 to 17-II-06 inclusive		Analysis of records of 4000 plague-infected rats during this period	
21867 were <i>M. decumanus</i>	70 %	Alive when brought for examination	0·82 %
9307 were <i>M. rattus</i>	30	Dead „ „ „	99·17
5951 were sent to laboratory alive	19	<i>M. rattus</i>	15·4
25223 were brought dead to laboratory	81	<i>M. decumanus</i>	84·6
16002 were males	51	Males	58·92
15172 were females	49	Females	41·07
4675 were plague-infected	15		

<sup>1</sup> This group includes both *Mus decumanus* and *Nesokia bengalensis*. The occurrence of this latter species was not at the time recognised: subsequent experience shows that about 1 % of the “decumanus” are in reality *Nesokia*.

<sup>2</sup> Every rat brought to the laboratory for examination is weighed. We have arbitrarily fixed the weight of a young “*rattus*” as being under 70 grammes and that of a young “*decumanus*” as being under 100 grammes. Animals with weights above these limits were regarded as adults.



### III. CHARACTERISTIC APPEARANCES IN PLAGUE-INFECTED RATS RECOGNISABLE BY NAKED-EYE EXAMINATION.

- (1) *The presence of rigor mortis, subcutaneous congestion, subcutaneous haemorrhages, and subcutaneous oedema*<sup>1</sup>.

*Rigor mortis* is fairly often present in plague rats, and is somewhat characteristic, the limbs projecting stiffly in a distinctive manner from the body. It may persist even when putrefaction has begun, in the internal organs. It was noted in 26·5 % of the rats in Series I.

*Subcutaneous congestion* is not infrequently a well-marked feature. It may be general but in some cases is limited to the neighbourhood of the bubo. In Series I it was present in 30·5 % of the total while in Series II a note was made of its presence in 69 %, it was well-marked in 7 % and was absent in 23 % of the rats<sup>2</sup>. A peculiar purplish-red appearance of the muscles exposed by reflecting the skin of the thorax and abdomen is obviously due to the presence of congested vessels and, combined with the reddish-pink colour of the subcutaneous tissue, presents an appearance which arouses a strong suspicion of plague at the commencement of the examination.

*Emaciation* was very rarely observed by us and is certainly not typical of plague. It may be said indeed that when a rat shows emaciation, and has lesions such as abscesses or septic lung conditions, the chances are greatly against it being plague.

*Subcutaneous haemorrhages* were noted in 40·5 % of the rats in Series I. In 18·5 % the haemorrhages were situated in the submaxillary region, and were associated with the occurrence of a bubo in this region, while in 8 % subcutaneous haemorrhages were noted in the submaxillary region although the bubo was in another situation or occasionally absent altogether. The general statement may be made that when present these haemorrhages are most frequently to be found in the submaxillary region. This depends doubtless upon the fact that haemorrhages are seen generally in the neighbourhood of buboes and that, as will appear later, buboes in rats are most often found in the neck. The next

<sup>1</sup> Unless special mention is made to the contrary all the observations in this paper refer to naturally infected rats.

<sup>2</sup> Subcutaneous congestion may manifest itself in a reddish hue of the skin before the rat is dissected. This is especially visible on the plantar surface of the hind feet which have a pink appearance. The sign is not however absolutely constant nor reliable, since it may not be observed in rats which are plague-infected and may occur in conditions other than plague.

common situation for these haemorrhages is the region of the flank. In young and medium sized rats especially they may be very widespread. We have never observed them in any rat which was not plague-infected. It is a matter of interest that they rarely occur in guinea-pigs infected either experimentally or naturally.

A general *oedema* of the subcutaneous tissue is a feature rarely met with in plague rats. When oedema is present it is usually limited to the region of the bubo. Thus in Series I cervical oedema was present in 10% of the cases. This contrasts with what is found in experimentally infected guinea-pigs in which general subcutaneous oedema is a very characteristic feature.

(2) *Changes in the lymphatic glands—buboes.*

If a dissection is made of a healthy rat the only glands which are large enough to be easily seen are those forming the crescent embracing the salivary glands in the submaxillary region, and the elongated retroperitoneal glands on each side of the middle line in the lower part of the abdomen. For the sake of brevity we will refer to the latter as "pelvic" glands.

In a septicaemic plague rat the glands in any region of the body may be enlarged and congested. Even when a primary bubo is present, secondarily enlarged glands may be found in a different situation. Thus the inguinal glands are not infrequently slightly swollen and congested, and may be surrounded by a characteristic radiating appearance due to an injection of the blood-vessels leading to and from the glands. Enlarged glands of this nature must be sharply distinguished from primary buboes. In the following description the use of the word bubo is restricted to mean a primary bubo and not these secondary glands.

The Austrian Plague Commission in their valuable account of the pathology of the lymphatic system in human plague make a distinction between primary buboes of the second order, *i.e.* glands in the neighbourhood of the primary bubo which have been directly infected from it, and secondary buboes which derive their infection from the blood when a septicaemia supervenes. In rats one occasionally finds both the inguinal and pelvic glands converted into primary buboes, the latter having obviously been infected by way of the lymphatics from the inguinal buboes. Such a lesion conforms to the description of a primary bubo of the second order.

## DESCRIPTION OF PLATE VII.

Fig. I. Healthy rat to be contrasted with plague-infected rat.

Fig. II. Plague-infected rat. A composite picture illustrating some of the common naked-eye pathological changes found in various organs and tissues in a plague-infected rat. (All the changes illustrated are rarely met with in a single specimen.)

Note (a) Marked subcutaneous congestion causing a peculiar pink appearance of the tissues which contrasts with the condition found in a healthy rat.

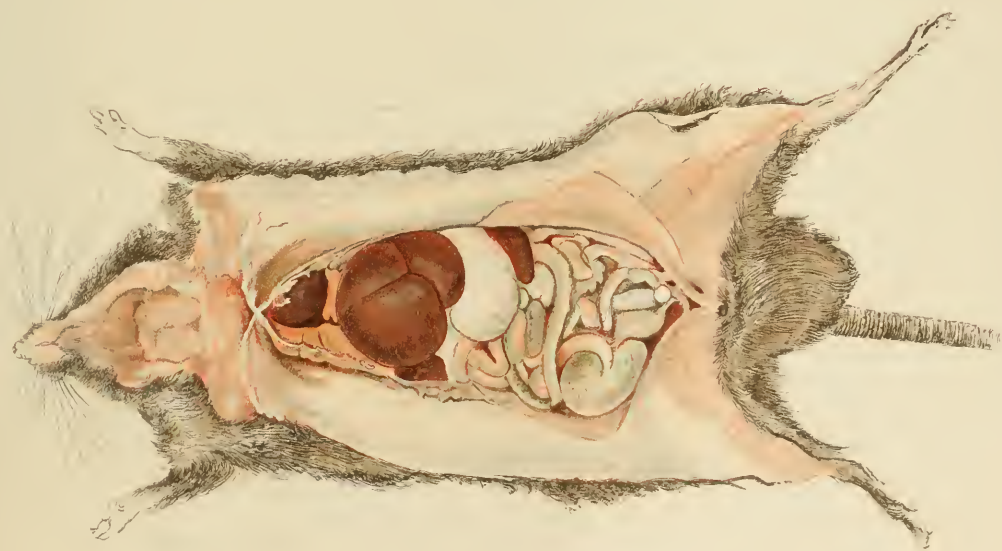
(b) Submaxillary bubo; the gland has been dissected out and defined for the purpose of illustration.

(c) Subcutaneous punctate haemorrhages most frequently found in the neck.

(d) Marked congestion and haemorrhages in the thoracic cavity especially in the lungs.

(e) Advanced stage of "mottled" and "granular" liver.

(f) Enlarged and congested spleen.



HEALTHY RAT





Occasionally the primary bubo is seen in the first stage of enlargement and congestion, showing haemorrhagic points when cut across. It may be distinguished from a secondary gland by the surrounding infiltration with perhaps haemorrhages in the subcutaneous tissue overlying it. Infiltration in the neighbourhood of the bubo extending into the subcutaneous tissue is indeed a highly characteristic feature of a bubo in any stage of its development. A localised subcutaneous oedema is sometimes observed. The presence of subcutaneous haemorrhages in the proximity of the bubo may often be noticed and these are frequently associated with marked congestion of the surrounding tissues.

A bubo feels hard when cut across though it has not the tough consistence of a normal gland. The contents of the latter are not easily squeezed out by pressure, whereas in a bubo the substance of the gland is readily broken down by slight pressure with the forceps.

TABLE III.

*Showing the occurrence and distribution of buboes in 4000 rats in Series II.*

Buboes in single situation only	=	2923	=	73.05 %
Multiple buboes	=	467	=	11.67
Bubo absent	=	610	=	15.25

*Of the buboes in single situation.*

Neck	=	2194	=	75
Axilla	=	440	=	15.1
Groin	=	178	=	6.1
Pelvis	=	111	=	3.8

TABLE IV.

*Showing order of frequency of various combinations of buboes in 467 rats with multiple buboes in Series II.*

Groin + axilla	in	32.3 %	(151)
Neck + axilla	„	28.2	(132)
Neck + groin	„	12.6	(59)
Neck + axilla + groin	„	7.4	(35)
Groin + pelvis	„	7.1	(33)
Groin + pelvis + axilla	„	4.9	(23)
Neck + pelvis	„	2.9	(14)
Axilla + pelvis	„	1.29	(6)
Neck + groin + pelvis	„	1.07	(5)
Neck + axilla + groin + pelvis	„	1.07	(5)
Neck + axilla + pelvis	„	0.85	(4)

Note :—Of the rats with multiple buboes 54.5 % had a bubo in the neck.

The typical appearance of a bubo on section is that of necrosis affecting first the medullary portion of the gland, and gradually spreading outwards so that ultimately the glands may be converted into a mass of necrotic tissue enclosed within the capsule. The central portion has consequently a gray appearance, or in a somewhat later stage contains a yellowish cheesy material. Rarely, in a still more advanced stage, the centre has broken down into a rather dry—still more rarely a liquid—purulent material. Buboes with greenish liquid pus are not typical of plague, and those examined specially have not proved to be plague.

At times one finds but little surrounding congestion of the tissues and the bubo itself may have a yellowish-white colour. Such a tissue offers a greater resistance than a normal gland when cut across. Microscopical examination of a bubo of this character reveals the presence of swarms of plague bacilli. Occasionally when a suspicious gland is cut across a creamy fluid exudes which on microscopical examination is found to consist of degenerated leucocytes, cellular debris, and masses of plague bacilli.

There is generally little difficulty in recognising a bubo simply on account of its relatively large size and from the fact that it causes a prominent swelling, *e.g.* in the submaxillary region where several buboes may be fused into a large mass. In many instances however the existence of a bubo in the neck may easily be overlooked for the reason that there is not much apparent swelling even when the neck glands are exposed. The glands in this region should always be arranged in their natural relations, and cognizance taken of the slightest asymmetry. Any suspicious gland should then be dissected out and cut across in several directions. The cut surface may show appearances suggestive of necrotic change and if so a smear should be prepared for microscopical examination. Indeed we made it a practice to cut into the neck glands of every rat examined. A bubo in the neck is sometimes readily found by probing with forceps in the region of the glands. Here it may be detected as a hard nodule like a pea.

Enlarged and congested glands in the groin and axilla ought to be incised and examined in the same way—a yellowish centre, if only the size of a pinhead, speaking strongly in favour of such a gland being a primary bubo.

Axillary buboes may readily be passed over when small, and especially if they are flattened and lie parallel to the inner surface of the arm under the insertion of the pectoral muscle into the humerus.

A routine practice should therefore be adopted of cutting through these muscles in the axilla.

It may be mentioned here that a common and marked feature of a bubo when examined microscopically is the presence of more or less numerous involution forms. Although secondarily enlarged glands may contain numerous bacilli these typically have the normal bipolar appearance.

(3) *Characteristic appearances in the abdominal organs.*

*The Liver.* The liver may show all degrees of "fatty"<sup>1</sup> change. In the early stages the lobules are clearly demarcated and this, combined with the yellowish appearance of the parts affected contrasting with the reddish colour of the congested areas, constitutes a characteristic picture which—perhaps inadequately—we have been accustomed to describe by the term "mottling." In some instances an extreme degree of "fatty" change is seen. In such a case the liver has a pink tinge, its surface presenting a uniformly smooth appearance and showing no sign of any division into lobules. The whole organ gives the impression of being modelled in wax with the upper surface peculiarly dome-like and the edges sharply defined. It has lost the normal tough resilient consistence, so that it pits on pressure and somewhat easily cracks on bilateral pressure especially when putrefaction has begun. This condition has not been seen in fresh rats other than those which were plague-infected, but in putrid rats an appearance very similar to it, or even indistinguishable from it, is rarely encountered when plague can with certainty be excluded.

Another condition frequently met with in the liver, and one of the greatest importance in diagnosis, is the occurrence of small necrotic foci scattered over its surface and throughout its substance. Tidswell makes mention of "small white points" in a few plague rats examined by him. Skschivan also from his description of several plague rats evidently recognised a similar appearance. We have been in the habit of referring to such a condition as "granular" liver. The gray or whitish granules are most easily observed on the surface; they are typically of the size of a pin's point and give the surface of the organ a stippled appearance as if dusted over with gray pepper. They are invariably discrete and in this respect contrast with the "mottled"

<sup>1</sup> The term fatty is used in reference to the naked-eye appearance only which strongly suggests an excess of fat. Microscopically however the appearance is found to be due to necrosis of the liver tissue.

liver in which there is no well-defined margin to any of the affected areas. They may be so small that only the closest scrutiny of an experienced observer will detect them. When larger the granules are of a yellow colour and vary somewhat in size. When well marked and closely set together they are always uniformly scattered throughout the liver substance, but if faintly marked and very few they may be confined to one lobe or to the edges only of the lobes. In some instances the necrosis assumes the appearance of a delicate gray network enclosing in its meshes the lobules which appear reddish from the presence of congested vessels. In a typical specimen the granules are not raised above the surface of the liver. Very exceptionally this does happen as in the case of an adult *M. rattus* where the liver is described as being "full of small yellowish white necrotic nodules about the size of a mustard seed, those on the surface being raised above the liver tissue that remained." On section the organ was found to be almost entirely replaced by the necrotic deposits, hardly any liver tissue being left. In this case a few typical bacilli only were seen microscopically but a pure culture of *B. pestis*, which gave good stalactites, was obtained from the heart-blood.

This granular condition of the liver is fairly often met with in experimentally infected rats which die about 48 hours after inoculation. The longer the interval between inoculation and death the better marked is the granulation. In a few instances rats killed on the 18th day have shown coarse granulation of the liver with very few plague bacilli present in the smears. With regard to the frequency of its occurrence it may be mentioned that it was noted in 58% of the rats in Series II. It is occasionally found in a liver which shows "fatty" changes. Even in putrid rats the granules may be recognised as gray points standing out on a black background.

Other pathological conditions met with in the liver in plague rats may be said to be neither constant nor characteristic. Haemorrhages under Glisson's capsule are seen relatively seldom. Enlargement and congestion of the liver, which some writers seem to consider noteworthy signs, are in our view of very little value.

*The Spleen.* The spleen of a plague rat is typically of firm consistence with a moulded appearance, so that it lies over the stomach in its natural relation to that organ instead of collapsing like a soft normal spleen. Its firm consistence probably accounts for the fact that a good smear is obtained from it on a slide with greater ease than from a normal spleen. Granules or nodules may be very well marked (*e.g.* the



size of a millet seed) and may be confluent. Sometimes a relatively large wedge-shaped portion of the spleen is converted into a cheesy mass in which plague bacilli can be found. A false appearance of granulation is often seen in normal spleens and is doubtless due to the Malpighian bodies showing through the semi-transparent capsule. Apart from this we have never seen a nodular condition except in a plague rat. Analysis of the records of 200 plague rats examined during December 1905 showed that 111 of the livers were granular (55·5 %), while the spleen was granular in 9, *i.e.* 4·5 %. In very rare instances the spleen contains granules although none are to be found in the liver.

Apparent enlargement and congestion of the spleen are of little importance for purposes of diagnosis. The spleen, especially in *M. decumanus*, is often much enlarged although the other organs are apparently normal; such a spleen is usually soft and flabby.

*The Kidneys.* The kidneys and the suprarenal capsules are often congested. Minute subcapsular haemorrhages are fairly often present *viz.* in 8·5 % of Series I. The kidneys frequently show "fatty" changes, sometimes appearing quite yellow. A granular condition of the organ is an extreme rarity although occasionally it has been noted.

*The Stomach and the Intestines* usually show no characteristic change. The latter may be acutely congested but subserous haemorrhages are rarely present, contrasting in this respect with plague guinea-pigs in which they are a common and striking feature. Haemorrhages are somewhat rarely seen under the peritoneal coat of the stomach.

Abundant peritoneal effusion is a rare occurrence though slight effusion may be seen, the serous surface having a moist look.

#### (4) *Characteristic appearances in the organs in the thorax.*

*Pleurae and Lungs.* Haemorrhages occur fairly often in the lungs and pulmonary pleurae but we have never seen them in the parietal layer of the pleurae.

The presence of *pleural effusion* is a very characteristic feature and one which we consider of great value in diagnosis. The effusion is typically quite clear and may be so abundant that when the sternum and portions of the ribs are reflected the heart and lungs appear to be floating in a bath of straw-coloured fluid which overflows, forming a pool in the axilla. It may sometimes be blood-stained. In Series I it occurred in 73·5 % of the rats while in 9 % it was abundant. In



TABLE V.

Serial No.	Date of examination	Species, sex, weight	Naked-eye appearances of lungs	Microscopical examination of lungs	Other post-mortem appearances	Microscopical examination of other organs	Confirmatory tests
1	9/4/06	Male <i>M. decumanus</i> 225 gms.	Left lung and right lower lobe consolidated; pleural effusion	Very numerous <i>B. pestis</i>	Right submaxillary bubo containing yellowish soft pus, no subcutaneous congestion; suspicious faint granulation in liver	Heart-blood—clumps of plague-like bacilli Spleen—0 Bubo—0	Subculture from bubo gave good stalactites
2	11/4/06	Female <i>M. decumanus</i> 350 gms.	Intensely congested with right lower lobe pneumonic; marked pleural effusion	Clumps of plague-like bacilli	Rigor mortis; no primary bubo; marked subcutaneous congestion; liver and spleen typically granular; haemorrhages around calices of kidneys; subcutaneous haemorrhages	Heart-blood—0 Spleen—0 Liver—0	Pure culture of <i>B. pestis</i> from lungs. Cultures from liver and spleen sterile. Culture from heart-blood contaminated
3	12/4/06	Male <i>M. decumanus</i> 300 gms.	Congested with right lower lobe consolidated; pleural effusion; haemorrhages in lungs	Swarms of plague-like bacilli	Rigor mortis; no primary bubo; slight subcutaneous congestion; liver mottled; spleen congested	Heart-blood—0 Spleen—0	Culture from lungs contaminated but showing plague-like growth, subculture pure plague. Spleen culture sterile; liver and heart-blood cultures contaminated
4	14/4/06	Male <i>M. rattus</i> 100 gms.	Deeply congested; lower lobe of right and left lungs consolidated; haemorrhages in lungs; pleural effusion	Swarms of plague-like bacilli	Rigor mortis slight; no primary bubo; subcutaneous congestion; liver mottled; subcutaneous haemorrhages	Heart-blood—a few suspicious bacilli amongst many putrefactive Spleen—0	Cultures of heart-blood, spleen, lungs all contaminated
5	16/4/06	Female <i>M. decumanus</i> 410 gms.	Deeply congested; left lung consolidated and showing gray hepatisation; haemorrhages in lungs; marked pleural effusion	Swarms of plague-like bacilli	Rigor mortis; left cervical bubo; very slight subcutaneous congestion; liver mottled	Heart-blood—a few doubtful <i>B. pestis</i> Spleen—0 Bubo—a fair number of <i>B. pestis</i>	Thick growth of plague in culture from lungs. Liver culture sterile
6	16/4/06	Male <i>M. decumanus</i> 300 gms.	Deeply congested; right lower lobe consolidated; haemorrhages in lungs; pleural effusion	Swarms of plague-like bacilli	No primary bubo; anterior mediastinal gland enlarged, congested; marked subcutaneous congestion with haemorrhages; liver mottled	Heart-blood—0 Spleen—0 Mediastinal glands—a few <i>B. pestis</i>	Thick growth of plague in culture from lung. Liver culture sterile
7	17/4/06	Female <i>M. decumanus</i> 280 gms.	Deeply congested; right lower lobe consolidated and patches of consolidation in left lung; pleural effusion	Swarms of plague-like bacilli in consolidated areas	No primary bubo; very marked subcutaneous congestion; liver granular	Heart-blood—0 Liver—0 Spleen—0	

Series II its presence was observed in 72%, it was noted as being abundant in 6.9% and it was absent in 28% of the rats.

*The Lungs* vary considerably in appearance and as a rule present nothing characteristic. They may exhibit a patchy congestion but in some cases they appear quite pale. Compared with guinea-pigs granules in the lungs of rats rarely occur—only 2.5% of the rats showing them in Series I.

An interesting feature somewhat rarely met with in plague rats is a pneumonia which is decidedly lobar in character. The details of seven cases have been collected in Table V. It will be observed that the lower lobe of the right lung was consolidated in six out of the seven rats, and that a double pneumonia was present in three. It will further be noted that microscopical examination of the lungs revealed very numerous plague-like bacilli (which were verified by culture in some of the cases) although relatively few or no bacilli were seen in the other organs. In two a submaxillary bubo was present, but the condition of the others leaves little doubt that they are instances of a typical primary pneumonia. We have observed pneumonic lungs in all stages including typical red and gray hepatisation and even apparent resolution. Portions of consolidated lungs sank when placed in water. 1000 plague rats not included in Series II were examined for the special purpose of noting the frequency of this condition with the result that six cases were found, viz. those numbered 2 to 7 in the table.

*The Heart.* The pericardium fairly often contains a clear fluid and epicardial haemorrhages occasionally are seen. The vessels on the surface of the heart frequently have an injected appearance. The walls are relaxed with the right cavities usually engorged with blood and the left empty.

#### IV. THE VALUE OF CERTAIN CHARACTERISTIC POST-MORTEM FEATURES IN THE DIAGNOSIS OF PLAGUE RATS, INCLUDING THOSE WHICH HAVE UNDERGONE PUTREFACTION.

A recapitulation may be conveniently given under this head of what we consider the most important post-mortem features for purposes of diagnosis.

The presence of a typical bubo is the most important sign of plague in rats.

The next important sign is the condition we have described as "granular" liver. In our experience this condition is not met with in rats other than those that are plague-infected. The spleen is a much less important organ for diagnostic purposes than the liver—in this respect, indeed, the latter takes the place of the spleen in guinea-pigs.

Haemorrhages, both subcutaneous and in the organs, are very suggestive features. They occurred somewhere or other in no less than 54% of the rats in Series I. We have already noted that so far as our experience goes subcutaneous haemorrhages constitute a most important sign of plague in rats.

Again an abundant clear pleural effusion of itself goes a long way towards establishing a diagnosis of plague.

In putrid rats, at least three of these signs may persist and when recognised are of the greatest assistance, viz. a bubo, granular liver and pleural effusion.

Table VI has been constructed in order to show the frequency of occurrence of most of the characteristic naked-eye features of the rats included in Series I.

TABLE VI.

*Showing frequency of occurrence of certain characteristic post-mortem features in the rats included in Series I.*

Post-mortem appearance or lesion	100 <i>M. rattus</i>	100 <i>M. decumanus</i>	Percentage of total
Rigor mortis ... ..	27	26	26·5
Subcutaneous congestion (including sub-maxillary) ... ..	22	39	30·5
Subcutaneous haemorrhages ... ..	44	37	40·5
Submaxillary haemorrhages with bubo ...	17	20	18·5
"                    " (bubo absent or in another situation) ...	7	9	8
Cervical oedema ... ..	9	11	10
Fatty liver ... ..	59	50	54·5
Granules in the lungs ... ..	1	4	2·5
"            " kidneys ... ..	0	1	0·5
Pleural effusion ... ..	73	56	64·5
Abundant effusion (included in above) ...	11	7	9
Haemorrhages in lungs and pleurae ...	16	32	24
"            " kidneys and suprarenals	5	12	8·5
"            " epicardium ... ..	2	5	3·5
"            " stomach ... ..	4	0	2
"            " intestine ... ..	0	1	0·5

V. THE OCCURRENCE OF PLAGUE-LIKE DISEASES AMONG RATS.

During sixteen months' continuous rat examination in Bombay, involving the scrutiny of 150,000 animals of which 19,000 were infected with plague, no disease of the rat has been met with which caused any material difficulty in diagnosis<sup>1</sup>.

VI. REMARKS ON THE RESULTS OF MICROSCOPICAL EXAMINATION.

The importance of the results which have been obtained by us from an analysis of this method of examination relates chiefly to the question of diagnosis.

For staining carbol-thionin blue was used invariably in the routine examinations. This has a certain value as a differential stain in that plague bacilli appear more faintly coloured than adventitious organisms. It brings out to advantage the typical bipolar appearance of *B. pestis*. Very rarely the bacilli in the organs assume the form of a small coccobacillus closely resembling the organism of fowl cholera and causing some doubt as to their real nature.

With regard to the presence of involution forms 56·6 % of the buboes in Series I showed them, while in the same number of spleen preparations examined they were found in only 12 %. In the spleen they occur perhaps most frequently in association with putrefactive organisms. They have never been observed in the heart-blood<sup>2</sup>.

Sometimes in rats which give evidence of a relatively chronic form of plague, with well-marked granules in the liver, the bacilli are not uniformly distributed over the preparation, but are present in the form of characteristic clumps. Clumps of bacilli were seen in 9·5 % of the spleen smears in Series I. They rarely occur in the heart-blood, having been seen once only in this series. When in clumps the bipolar appearance is much less often observed than when the organisms are uniformly distributed in the smear, the contents of the bacilli usually appearing very finely and uniformly granular.

<sup>1</sup> It is perhaps worth recording that the leprosy-like disease of rats due to acid fast bacilli described by Stefansky (*Centralblatt für Bakt.*, xxxiii. 1903, p. 481) and Dean (*This Journal*, vol. v. p. 99) has been met with in *M. decumanus* in Bombay and in *M. rattus* in the Punjab.

<sup>2</sup> They have been observed in the heart-blood of plague guinea-pigs, and very rarely (namely on two occasions) in the heart-blood of experimentally infected rats.

The general value of the method of microscopical examination is sufficiently indicated by the fact that in 75% of the total rats in Series I numerous plague bacilli were seen either in the heart-blood, spleen, or bubo of each rat, or, if not very numerous in the bubo, involution forms were present.

As to the comparative value of the three tissues usually examined, there can be no doubt that the bubo gives a better chance of finding plague bacilli than the spleen, and the spleen than the heart-blood. Thus, out of 150 rats with buboes in Series I, numerous *B. pestis* were noted in 104 preparations of the buboes, 70 preparations of spleens and only 27 preparations of the heart-blood. Results tending in the same direction will be found in the appended statement of an analysis of the microscopical examination of 1000 rats in Series II (Tables VII, VIII,

TABLE VII.

*Analysis of results of microscopical examination of 1000 rats with buboes in Series II.*

	No <i>B. pestis</i> seen	Few <i>B. pestis</i> seen	Numerous <i>B. pestis</i> seen
Heart-blood	13.5 %	53.4 %	33.1 %
Spleen	9.9	17.3	72.8
Bubo	0.9	7.4	91.7
No <i>B. pestis</i> seen in smears of bubo, spleen and heart-blood			0.4 %
<i>B. pestis</i> seen in smears of bubo only		...	6.6

TABLE VIII.

*Analysis of 37 putrid rats out of the 1000 rats with buboes in Series II.*

	No <i>B. pestis</i> seen	Few <i>B. pestis</i> seen	Numerous <i>B. pestis</i> seen
Heart-blood	24.3 %	37.8 %	37.9 %
Spleen	29.7	13.5	56.8
Bubo	5.4	13.5	81.1
<i>B. pestis</i> not seen in any of the three smears			5.4 %

TABLE IX.

*Analysis of results of microscopical examination of 100 M. rattus and 100 M. decumanus in Series I.*

	<i>M. rattus</i>			<i>M. decumanus</i>		
	None	Few	Numerous	None	Few	Numerous
Heart-blood	19 %	48 %	29 %	29 %	59 %	7 %
Spleen	8	26	64	14	51	31
Bubo	1.3	24	72	8	20	69



TABLE X.

*Comparison of results of microscopical examination of Series I (combined percentages of 100 M. rattus and 100 M. decumanus) and 1000 rats with buboes in Series II.*

	Series I	Series II
Heart-blood=0 or doubtful	28·5 %	13·5 %
Heart-blood=few	53·5	53·4
Heart-blood=numerous	18	33·1
Spleen =0 or doubtful	14	9·9
Spleen =few	38·5	17·3
Spleen =numerous	47·5	72·8
Bubo =0 or doubtful	7·4	0·9
Bubo =few	22	7·4
Bubo =numerous	70·6	91·7

Note:—Under the term doubtful are included those cases in which microscopical examination did not reveal the presence of plague bacilli with reasonable certainty.

and X). Even in a very putrid rat the bubo may show many plague bacilli, frequently with involution forms in addition, but with relatively much fewer putrefactive organisms than in the smears of the spleen or of the heart-blood. In a suspicious bubo showing no plague bacilli the presence of degenerated leucocytes and cellular débris serves materially to strengthen the suspicion of plague.

#### VII. ON THE RELATIVE VALUE OF THE METHODS OF NAKED-EYE AND MICROSCOPICAL EXAMINATION IN THE DIAGNOSIS OF RATS SUSPECTED OF BEING PLAGUE-INFECTED.

Any value which our epidemiological inquiries into plague in Bombay may possess necessarily hinges to no small extent upon the accuracy of the daily returns of the plague-infected rats. For this reason it became a matter of importance to acquire as thorough a knowledge as possible of the appearances presented by the plague rats, and of the reliable methods for the diagnosis of rats suspected of being plague-infected. It is obvious, moreover, that especially in the plague season, when as many as 200 rats were returned daily as plague-infected, some system of examination had to be adopted which should give the best results without needless expenditure of time and labour. A description of the methods carried out by us in the daily rat investigation, in so far as they are concerned with the recognition of plague rats, may not be considered out of place here.

At the beginning of the investigation in July 1905, and for several months thereafter, a smear of the spleen of every rat brought to the laboratory was examined microscopically, the post-mortem appearances of the rats being also noted. In addition to this, the spleen or other organ of every rat which was suspected of being plague-infected either by macroscopical or by microscopical examination, and even of those which were regarded with certainty as plague rats, was inoculated cutaneously into a guinea-pig. After a complete and careful examination had been made of 50 plague rats verified in this manner, the procedure was so far modified that only those rats which offered difficulty in diagnosis were submitted to the animal test. The routine microscopical examination of the spleen smears was carried out during the off season, but later, having regard to our increasing experience in diagnosis by macroscopical examination alone, it was decided to forego the former method. The system adopted at this stage may be briefly described.

The rats were cut open by three soldiers attached to the Plague Research Laboratory. These men proved to be very intelligent assistants—their knowledge of the appearances of plague rats as the result of an extensive experience being remarkably accurate. Rats recognised as plague by them were sent to a room in another part of the building, where a detailed examination was carried out by members of the staff. Moreover, two or three members of the staff examined carefully every rat passed over by the soldiers, and any rat with suspicious features was sent for further examination to the room referred to. Thus, a detailed examination was made of every plague rat and every rat suspected of being plague—a record being made of the post-mortem appearances and of the results of microscopical examination of the heart-blood, spleen, and bubo if present. The returns of plague rats were compiled after a consideration of the results of the complete examination. In cases where the diagnosis remained uncertain the organs were inoculated cutaneously into a guinea-pig and, if thought advisable, cultures were made. Nearly 5000 plague rats were examined in this way during the early period of the epizootic. It then became apparent that such a detailed examination might be curtailed without substantially affecting the accuracy of the returns. The method was accordingly limited to a careful checking of every rat by two or three trustworthy and highly trained observers. Only in the case of suspicious rats were the organs examined microscopically.

In order to discover whether any serious error had crept in from the

omission of the microscopical examination of every rat brought to the laboratory, and whether the naked-eye method of examination alone might be considered reliable, it was arranged to carry out a test extending over a fortnight with this object in view. The plan pursued was as follows:—

The soldiers put on one side the rats they believed to be plague-infected, and if they regarded any as suspicious a note to this effect was made on the card affixed to the rat. At the same time the members of the staff whose duty it was under ordinary circumstances to observe the post-mortem appearances recorded their opinion of every rat examined by them. To three experienced microscopists the task was allotted of examining preparations from the spleen of all the rats; they likewise recorded their opinion of every slide examined. Finally, a member of the Commission compared the results arrived at by the two methods. It ought to be explained that in order to avoid any bias which might arise from their inclusion amongst the spleen smears, preparations from buboes were not given in the first instance to the microscopists. An opportunity was, however, afforded of examining smears of buboes or of the heart-blood if the original spleen smear proved negative on repeated examination.

An endeavour was made by the individual superintending the test to be impartial in his appraisal of the results, though it must be admitted that no criterion is available on which to base a final decision in every instance since even the cutaneous test may fail in certain cases. It may be added that when any of the men engaged in carrying out the test disagreed in his opinion with the others he was permitted to have the suspected material inoculated into a guinea-pig. This was done indeed in every case in which there was a wide divergence of opinion as regards the diagnosis. A detailed analysis of the results of the last six days of the test has been arranged in the following Tables (XI—XIV).

A review of this analysis leaves no room for doubt that for purposes of diagnosis naked-eye examination by a competent observer is more satisfactory than microscopical examination alone. It is somewhat remarkable that in a single instance only was a plague rat diagnosed by microscopical examination which the observers of the post-mortem appearances failed to recognise, *i.e.* 0·7 % of the total number of plague rats. On the other hand six rats with plague bacilli in the spleen smear were overlooked by the microscopists, and in seven rats no plague bacilli were found microscopically in any of the organs, *i.e.* 13

rats were missed out of a total of 131, viz. nearly 10%. Naturally in isolated cases both methods must be employed, but the results clearly show that the omission of the routine microscopical examination of every rat in an investigation conducted on a large scale does not necessarily impair the accuracy of the work, while the saving of labour is of course very great.

It will readily appear from Tables XIII and XIV that the chief difficulty which is encountered in diagnosis by either of the methods

TABLE XI.

*Showing analysis of results of experiment to compare macro- and microscopical methods of diagnosis.*

Date	Total No. of rats examined	Total No. returned as plague	Plague rats missed by soldiers	Plague rats missed by expert observers of P.M. appearances	<i>B. pestis</i> present in spleen smear but overlooked by microscopists	<i>B. pestis</i> not seen in spleen smear but seen in smear of other organ or in bubo	<i>B. pestis</i> not seen in any organ microscopically
21/5/06	205	25	—	—	2	2	1
22/5/06	165	20	—	—	—	—	1
23/5/06	195	17	—	—	—	1	2
24/5/06	171	23	—	—	2	—	1
25/5/06	223	21	—	—	1	2	2
26/5/06	214	25	1	1 <sup>1</sup>	1	3	—
Total	1173	131	1	1	6	8	7

<sup>1</sup> It may be explained that this rat was very putrid with an inguinal bubo which had already been opened by one of the soldiers. The appearance of the cut surface of such a bubo rapidly alters by exposure to air, and as this was the only suspicious feature present the failure to recognise the rat as being plague-infected is excusable. Moreover it had already been noted as "suspicious" by the soldier who dissected it.

TABLE XII.

*Giving an analysis of the diagnosis of the rats returned as plague which were diagnosed in the first instance either as "suspicious" or "plague" both by the Post Mortem observers and by the microscopists.*

Date	Total No.	Diagnosis of "plague" by post-mortem examination	Diagnosis of "suspicious" by post-mortem examination	Diagnosis of "plague" by microscopists	Diagnosis of "suspicious" or "very suspicious" by microscopists
21/5/06	20	20	—	17	3
22/5/06	19	18	1	15	4
23/5/06	14	13	1	13	1
24/5/06	21	17	4	15	6
25/5/06	16	16	—	13	3
26/5/06	20	19	1	16	4
Total	110	103	7	89	21



TABLE XIII.

*Giving details of the rats which were proved to be plague by cutaneous inoculation into guinea-pig.*

Serial No.	Date	Diagnosis of observers of post-mortem	Diagnosis of microscopists	Post-mortem features of rats
1	14/5/06	Suspicious	<i>B. pestis</i> not recognised	Semi-putrid; no primary bubo; subcutaneous congestion; spleen congested and firm; pleural effusion
2	14/5/06	Suspicious	<i>B. pestis</i> not recognised	Semi-putrid; no primary bubo; subcutaneous congestion; liver pinkish; lungs deeply congested; pleural effusion
3	14/5/06	Suspicious	<i>B. pestis</i> not recognised	Semi-putrid; no primary bubo; slight subcutaneous congestion; liver pink; lungs deeply congested with slight effusion
4	15/5/06	Suspicious	Plague	Putrid; liver pale; guinea-pig died in four days
5	16/5/06	Plague	<i>B. pestis</i> not recognised	Putrid; right cervical bubo; coarsely granular liver and spleen; haemorrhages in lungs with slight pleural effusion
6	16/5/06	Very suspicious	<i>B. pestis</i> not recognised	Putrid; right cervical bubo?; spleen firm and moulded; lungs deeply congested with pleural effusion
7	19/5/06	Suspicious	Plague	No primary bubo; enlarged and congested secondary glands; subcutaneous oedema and congestion; organs congested; abundant pleural effusion
8	19/5/06	Suspicious	<i>B. pestis</i> not recognised	Putrid; no primary bubo; very slight subcutaneous congestion; liver pinkish and firm; lungs congested with pleural effusion; subcutaneous haemorrhages
9	21/5/06	Plague	<i>B. pestis</i> not recognised	Putrid; no primary bubo; lungs congested with pleural effusion
10	23/5/06	Suspicious	<i>B. pestis</i> not recognised	Semi-putrid; right axillary bubo?; slight subcutaneous congestion; liver pinkish; lungs congested; pleural effusion

*Summary of diagnosis in above Table.*

Diagnosis of "suspicious" by p.m.	=7	<i>B. pestis</i> not recognised by microscopists 8	
Diagnosis of "very suspicious" by p.m.	=1		Diagnosis of "plague" ... .. 2
Diagnosis of "plague" by p.m.	=2		

TABLE XIV.

*Showing analysis of diagnosis of 21 rats which did not prove to be plague-infected when inoculated cutaneously into guinea-pig.*

	Diagnosis by P.M. examination	Diagnosis by microscope
Not plague	9	7
Suspicious	5	7
Very suspicious	5	3
Plague	2	4

Note:—19 out of the 21 rats were more or less putrid.



arises from putrefactive changes masking the characteristic appearances in the organs. In this connection it may be convenient to give details of an experiment devised in order to test the ability of those in charge of the diagnosis of the rats in the routine examination to recognise putrid plague rats. A number of Bombay rats were inoculated under the skin of the back with a virulent culture of the plague bacillus. Twelve of these rats which died at about the same time were laid aside exposed to the air in a go-down along with a number of healthy rats freshly killed by chloroform. In all some 50 rats were thus exposed. The rats were labelled in such a way that they gave no indication to those examining them as to whether or not they had been inoculated. After being in the go-down for 48 hours, they were dissected and an independent opinion was given of the diagnosis from post-mortem and from microscopical appearances. As will be seen from Table XV extreme putrefactive changes had set in in most of the rats. The results of the experiment are briefly as follows:—of the 12 plague rats seven were diagnosed as plague by the observers of the post-mortem appearances, in addition two were noted as “suspicious,” while the remaining three had become so putrid that it was impossible to put forward any diagnosis. The signs which, although faintly marked (as is generally the case in artificially inoculated rats), gave a clue to the diagnosis of “plague” or “suspicious” were—an appearance of general congestion, some moisture in the pleural cavities, oedema in the axilla in a few (the bubo had already disappeared), and a somewhat firm consistence of the spleen and liver in others.

It would appear from this test, also, that no serious error can be ascribed to the naked-eye method of diagnosis in the direction of failing to recognise rats which show advanced putrefactive changes. As a matter of fact the number of rats brought to the laboratory for examination which showed such extreme putrefaction as those in the experiment just described is so small that it does not deserve to be taken into account.

Material from all these rats was inoculated cutaneously into guinea-pigs, but by an oversight the diagnosis attached to each rat and the results of the cutaneous tests were not correlated. Still, the fact that seven out of the 12 rats gave plague to guinea-pigs serves to strengthen the impression that even in a severe test of this description a reliable method for diagnosis is to be found in the cutaneous method of inoculation.

TABLE XV.

*Giving details of plague rats in the test for diagnosis of putrid rats.*

Test No. of rat	Extent of putrefaction Putrid and full of maggots	Microscopical examination of rat Smears of H.-B. and spleen putrefactive organisms, none plague-like	Cutaneous test in guinea-pigs Spleen and H.-B. inoculated. Guinea-pig died in 5 days of typical plague
2	Very putrid; organs almost entirely eaten away by maggots except those in thorax	H.-B. and spleen—all putrefactive organisms, none plague-like	Heart inoculated. Guinea-pig chloroformed on 5th day: plague
8	Fairly fresh; liver pink and firm; subcutaneous congestion; lungs congested with pleural effusion	H.-B.—many putrefactive; a few plague-like	Spleen inoculated. Guinea-pig chloroformed on 3rd day: plague
13	Similar to No. 8	H.-B.—some like typical plague; involution forms seen, many putrefactive	Heart inoculated. Guinea-pig chloroformed on 6th day: plague
30	Putrid	H.-B.—plague-like and many putrefactive bacilli	Spleen and H.-B. Guinea-pig chloroformed on 6th day: plague
34	Very putrid	H.-B. and spleen—many putrefactive, none like plague	Spleen and H.-B. Guinea-pig chloroformed on 6th day: plague
39	Putrid	Similar to rat 34	Spleen. Guinea-pig chloroformed on 6th day: plague
3	Very putrid and full of maggots	H.-B.—a few like plague, many putrefactive. Spleen—putrefactive only	Guinea-pig failed to take plague
16	Very putrid and destroyed by maggots except hind limbs, muscles of which were soft and pulpy	H.-B. Spleen—putrefactive only—none like plague	Guinea-pig failed to take plague
24	Putrid and full of maggots	Similar to 16	Guinea-pig failed to take plague
41	Putrid	Similar to 16	Guinea-pig failed to take plague
49	Putrid	H.-B.—a few involution + putrefactive bacilli. Spleen—putrefactive only	Guinea-pig failed to take plague

*Summary of results obtained by different methods of diagnosis in this test.*

1. By p. m. examination 7 were considered to be plague; 2 as suspicious.
2. By cutaneous tests 7 were proved to be plague.
3. By microscopical examination 5 were considered as suspicious.

VIII. ON DIAGNOSIS BY THE CUTANEOUS METHOD OF INOCULATION  
WITH REMARKS ON THE VIRULENCE OF THE BACILLUS OF  
NATURAL RAT PLAGUE.

The following account of a somewhat extensive experience in the diagnosis by means of the cutaneous method in guinea-pigs of plague-infected rats or of rats suspected of being plague-infected is based principally upon an analysis of 330 consecutive examinations carried out according to this method, 150 of which proved to be plague. The rats formed part of the daily collections made in Bombay, and were sent to the laboratory for examination by the Commission during the period between July and December 1905. Plague in rats sent to the laboratory alive was a rare occurrence during this period, so that as a matter of fact in nearly every case the rats were dead when brought to the laboratory and showed varying degrees of freshness or in some cases of putrefaction. It must be remembered that the temperature in Bombay favours rapid putrefaction, thus adding greatly to the difficulties of diagnosis, especially by cultural methods. For the sake of simplicity a division has been made between those which might be considered "fresh," *i.e.* without obvious signs of putrefaction, and those which were decidedly putrid; thus the material inoculated was derived from 123 fresh rats and from 27 putrid rats.

It may be added that the majority of the rats belonged to the species *M. decumanus*.

Before commenting generally on the results, the technique adopted by us may be briefly described, together with the effects which follow inoculation of plague material into the skin of a guinea-pig.

The method was as follows. An area of skin about one inch square of the guinea-pig's abdomen is shaved with a sharp razor, no water nor soap being used. It is important to avoid the use of soap in shaving the skin, as there is good reason to believe that the chances of the guinea-pig dying acutely are thereby greatly diminished. The epidermis is partly removed in shaving, so that a raw, slightly bleeding surface is exposed. Pieces of the organ or organs selected for the test are then removed with sterile scissors and forceps, and rubbed, with some vigour, by means of the forceps, over the shaved area. This procedure is adhered to however putrid the material may be.

In the employment of the cutaneous method as a confirmatory test for rats diagnosed as plague or for rats suspected of being plague-

TABLE XVI.

Serial No.	Dilution	Method	Weight	Local reaction	Result
1	0·000001	Cutaneous	Increased steadily in weight	None	Recovered
2	0·000001	Subcutaneous	Gained weight till 6th day, then gradually lost weight till original weight on 12th day, thereafter gained weight	Small reaction noted on 8th day	Killed by $\text{CHCl}_3$ on 23rd day; P. M. very chronic plague
3	0·00001	Cutaneous	Gained weight	None	Recovered
4	0·00001	Subcutaneous	Gained weight till 5th day, then gradually lost weight till death	Slight reaction on 5th day	Died on 13th day; P.M. chronic plague
5	0·0001	Cutaneous	Gained weight till 5th day then lost weight	—	Died on 6th day
6	0·0001	Subcutaneous	Gained weight till 6th day. Final weight same as original	Reaction on 4th day	Died on 13th day
7	0·001	Cutaneous	Gained weight till 6th day, then lost weight. Final weight = original weight—50 grams	—	Died on 18th day
8	0·001	Subcutaneous	Gained weight till 6th day. Final weight = original weight—10 grams	Reaction on 3rd day	Died on 18th day
9	0·01	Cutaneous	Lost weight on 3rd day. Final weight = original weight—30 grams	—	Died on 9th day
10	0·01	Subcutaneous	Lost weight on 3rd day. Final weight = original weight—40 grams	Reaction on 2nd day	Died on 7th day
11	0·1	Cutaneous	Lost weight on 3rd day. Gained weight on 14th day	—	Killed by $\text{CHCl}_3$ on 23rd day; P.M. chronic plague
12	0·1	Subcutaneous	Lost weight on 2nd day. Final weight = original weight—50 grams	Reaction on 3rd day	Died on 6th day



infected it is important that the bubo, if present, should be rubbed in. It has been shown above that plague bacilli are more often found and, when present, are more numerous in the bubo of a plague-infected rat than in any other tissue.

The factors which influence the result of an inoculation of plague bacilli into an animal are various.

They are partly illustrated in Table XVI, which compares the effects of graduated quantities of virulent plague bacilli inoculated cutaneously and subcutaneously in a parallel series of guinea-pigs. The culture employed in the experiment was a subculture in broth of a strain recently isolated from the blood of a septicaemic patient. The dilutions were made in fresh normal urine, 0.25 c.c. of each dilution being used for each inoculation.

It is obvious first of all that the question of dosage is an important one. This is apparent in the case of guinea-pigs 1 and 3, which remained unaffected by cutaneous inoculation of small doses of virulent *B. pestis*, although subcutaneous injection of the same amount produced chronic plague in guinea-pigs 2 and 4. Again the influence of individual resistance of the animal experimented upon must explain the case of guinea-pig 11, which received the largest dose of the cutaneous series and which, nevertheless, lived for more than three weeks. Another factor not taken into account in this series, since the material used was the same throughout, is that of virulence of the bacilli inoculated. Yet another circumstance which must be reckoned with is the association with plague bacilli of other organisms in the material used for cutaneous inoculation, *e.g.* putrefactive organisms. This point will be adverted to later.

With regard to the subcutaneous series it will be observed that when a small dose is injected, the animal suffers from a chronic form of plague. In such cases it may continue to gain weight during the first five or six days and the local reaction is delayed even for a week. When larger amounts are given loss of weight is noticed on the second or third day, and at the same time a reaction may be felt in the glands near the site of injection. A very similar state of things seems to hold good in animals inoculated cutaneously, as will appear in the examples to be given shortly.

From the point of view of early diagnosis by the cutaneous method the appearance of a reaction at the site of inoculation and the existence of enlarged inguinal glands deserve attention. One or two actual instances will best illustrate this.



I. A guinea-pig was inoculated cutaneously in the usual manner with the spleen of rat 50237 on 23-XII-05. This rat was considered suspicious on detailed examination. No primary bubo was seen; the liver was granular; there was pleural effusion and subcutaneous haemorrhages were noted. Microscopical examination of the heart-blood and spleen showed no *B. pestis*. Twelve hours after inoculation the guinea-pig showed a slight skin reaction consisting of phlyctenules surrounded by a zone of redness. No bubo could then be felt. Thirty-six hours after inoculation the skin reaction was marked and small inguinal buboes could be felt. The guinea-pig died with typical signs of plague on the fifth day; the weight remained the same throughout.

II. A guinea-pig was inoculated cutaneously with the spleen and bubo of rat 49985 on 23-XII-05. The rat was putrid, but a right sub-maxillary bubo was found. Microscopical examination of the heart-blood and bubo showed no *B. pestis*, but a few suspicious bacilli were seen in a spleen smear. The detailed examination was considered suspicious of plague. The guinea-pig showed a skin reaction 12 hours after inoculation. A small inguinal bubo was felt for the first time on the 5th day—the skin reaction being then well marked. The animal was killed by chloroform on the 10th day, when axillary and inguinal buboes, containing typical plague bacilli were found on post-mortem examination. There was a very gradual increase of weight throughout, so that the final weight was 30 grams in excess of the original weight.

III. A guinea-pig was inoculated in the usual way with the spleen of rat 50750 on 26-XII-06. The rat was missed in the routine microscopical examination of the spleen smears, but was regarded as suspicious on detailed examination. There was no bubo; the liver was granular, and pleural effusion and subcutaneous haemorrhages were observed. Twelve hours after inoculation a skin reaction was noted, the guinea-pig, however, being well and active; 24 hours later, the inguinal glands on the left side were felt to be enlarged. The guinea-pig died in three days of typical plague. The final weight was 10 grams in excess of the original weight.

These cases exemplify a general experience, namely, that the cutaneous reaction is the earliest symptom, usually appearing about 12 hours after inoculation. If the disease is acute the inguinal glands can be felt to be enlarged 36 hours after inoculation, while in chronic cases the glands may be palpable only after the lapse of several days.

*Inoculation of fresh material.*

Proceeding now to the consideration of the material examined by us in the course of the routine diagnosis, a matter of considerable importance will be found in an analysis of the varying periods before death of the inoculated animals.

Table XVII, in which the facts have been arranged, makes it sufficiently clear that a large percentage of the guinea-pigs died acutely, that is in from two to four days. Thus, no less than 41% of the cases died on or before the 4th day, and 62% in five days. The largest percentage of deaths on any one day occurred on the 5th day, viz. 21.1%.

TABLE XVII.

Death in days	No. of guinea-pigs which died	
Death in 2 days	3	2.4%
„ 3 „	24	19.5
„ 4 „	23	18.7
„ 5 „	26	21.1
„ 6 „	18	14.6
„ 7 „	10	8.1
„ 8 „	2	1.6
„ 9 „	2	1.6
„ 10 „	2	1.6
Chloroformed after 10 days	4	3.2
Chloroformed in 10 days or before 10 days	9	7.3

Since the statement has been made (Klein, 1906) that plague in guinea-pigs following cutaneous inoculation almost invariably takes a subacute form (*i.e.* death in more than three days), we feel justified in claiming that the bacilli in our experiments causing acute death must have possessed a high degree of virulence. Nor can the objection be raised that the acuteness of the disease was due to a massive dose of bacilli. At least this cannot have been the case always as will be seen from a review of Table XVIII, in which the data have been classified with reference to three things—1st, the period before death of the guinea-pigs; 2nd, the microscopical examination of the material inoculated; 3rd, the results of the post-mortem examinations of the rats from which the material was derived. Study of this table leads to the conviction that in a large number of cases the material rubbed in contained highly virulent plague bacilli. This conclusion receives support from the fact that many of the organs when examined microscopically showed

either no plague bacilli or suspicious bacilli only, so that the quantity inoculated was presumably a small one, and yet the guinea-pigs died of acute or subacute plague. If a calculation of the results be made on the basis of the standard of virulence adopted by Kolle and Martini (1902) it will be found that at least 62% of the strains used in the experiments were fully virulent, *i.e.* caused death up to five days.

TABLE XVIII.

*Fresh material inoculated (114 experiments).*

Death in days	Microscopical examination of inoculated material					Post-mortem examination of rat		
	Suspicious bacilli	No <i>B. pestis</i> seen	A few seen	Fairly numerous	Numerous	Typical	Fairly typical or "suspicious"	Nothing typical of plague
Death in 2 days	—	—	1	—	2	3	—	—
„ 3 „	10	2	7	1	4	11	10	3
„ 4 „	9	3	2	4	5	15	4	4
	19	5	10	5	11	29	14	7 <sup>1</sup>
Death in 5 days	9	5	2	3	7	14	11	1
„ 6 „	3	3	2	5	5	12	6	—
„ 7 „	3	1	1	2	3	8	2	—
„ 8 „	—	—	—	—	2	2	—	—
„ 9 „	—	—	—	—	2	2	—	—
„ 10 „	1	—	—	—	1	1	—	1
Chloroformed after 10 days	—	—	—	1	3	4	—	—
	16	9	5	11	23	43	19	2

The only evidence pointing to an occasional diminution of virulence of the bacilli in natural rat plague may be drawn from the case of four guinea-pigs inoculated separately with material containing numerous *B. pestis*. One guinea-pig was killed by chloroform on the 10th day, two on the 11th day, and one on the 14th day after inoculation, all showing signs of chronic plague. The possibility of an unusual resistance to plague in these particular animals cannot, however, be altogether excluded.

An additional strong argument in favour of the view that the type of bacillus associated with natural rat plague is a virulent one lies in the

<sup>1</sup> The number of rats classed under this head would probably have been much reduced if these experiments had been made later in our experience of rat plague: see the results in Tables XI, XII, XIII and XV.

fact that out of 300 rats examined the inoculation apparently failed in three instances only of rats which were strongly suspected of being plague-infected. In further proof of the virulence of the bacillus of natural rat plague, we may point out that the cultures used to set alight the epizootics carried on by rat fleas in the go-down experiments were derived from naturally infected Bombay rats.

There is some indication that the variation of virulence for the guinea-pigs is an expression of variations of virulence for the corresponding rats. Table XVIII shows that the rapidity of death of the test guinea-pig varies inversely with the suggestiveness of the post-mortem appearances of the rat. Thus 14% of the inocula which killed guinea-pigs in four days or less were derived from rats which showed nothing typical of plague on section, while but 3% of those which produced death at a later period were derived from such indeterminate animals. Excluding races of very low virulence, it is probable that the degree of reaction (*i.e.* the degree to which the naked-eye post-mortem abnormalities are developed) varies inversely with the virulence of the infecting strain of bacillus, and that therefore the races most virulent to guinea-pigs were also most virulent to rats.

Before leaving the subject of virulence, attention may be called to the fact that the rats dealt with in Table XVIII were examined in the off-plague season, *i.e.* when only sporadic cases were occurring in rats and in men. The only statement founded on experiment relating to possible fluctuations in virulence of the bacillus of natural rat plague appears to be that made by Skschivan (1903) in a paper describing the epizootic in Odessa in 1901—1902. Our own experience receives confirmation from the observations of this writer, although it must be said that his conclusions are based upon the examination of only 32 rats. Skschivan concludes that the virulence of the bacilli does not become weakened by successive passages through rats, since, for example, one of the last plague rats found contained virulent bacilli.

These results, taken in conjunction, have an obvious bearing upon the question of the transmission of plague from rat to rat in nature, but they retain their significance only on the view that such transmission is of a more or less direct character, *e.g.* by entrance of the bacilli through an abrasion in the skin. Their import is considerably modified if the assumption be made that an alteration of virulence may be effected in an intermediate host, for example, in the stomach of the flea. Not only do the results affect the general problem of the transmission of plague in rats, but they may bear upon a more specific point, namely, the



seasonal periodicity of plague amongst rats. An opportunity will, doubtless, be afforded later of discussing this subject from every possible point of view.

It is evident from the examples given previously that a cutaneous reaction and the presence of inguinal buboes are outstanding features when the inoculation proves successful. Another symptom equally important is the loss of weight which occurs as the result of infection. When death takes place very acutely, the animal may even gain slightly in weight, otherwise there is a varying loss of weight depending upon the acuteness of the disease. The average daily loss of weight is seen in Table XIX to be the greatest in the case of guinea-pigs which die on the 5th day. An early decrease in weight then gives valuable indication that infection by plague bacilli is in progress. A striking contrast is afforded in the case of guinea-pigs in which infection has failed—the animal steadily gains weight from the beginning, and the skin abrasions rapidly heal.

TABLE XIX.

Death in days	No. of guinea-pigs whose weight was observed	Average daily change of weight
2	3	Gained 1·6 grams
3	19	Lost 5·2 „
4	16	„ 7·8 „
5	23	„ 8·4 „
6	18	„ 6·7 „
7	10	„ 7·2 „
8	2	„ 7·5 „
9	2	„ 8·3 „
10	2	„ 3·5 „

*Inoculation of putrid material.*

The value of the cutaneous test for plague is especially manifest in those cases where the material inoculated is derived from rats which have undergone putrefaction. The details of those selected for the purposes of this paper have been collected and arranged in Table XX.

We may note that the percentage of acute deaths (22 % by the 2—4 days' standard—40 % by the 2—5 days') is considerably less than in the series of fresh material. It must be admitted, however, that the percentages have been calculated on small numbers. It is possible that the discrepancy is explicable on the view that a small amount of *B. pestis* was inoculated on account of the presence of other organisms,



or it may be that the virulence of the plague bacillus is diminished by association with putrefactive organisms. The latter view is not, however, supported by the circumstances that very putrid material containing very few *B. pestis* killed some of the inoculated animals with the acute disease.

TABLE XX.

Death in days	Number of guinea-pigs which died
Died 2 days	0
" 3 "	2
" 4 "	4
" 5 "	5
" 6 "	3
" 7 "	4
" 8 "	3
" 9 "	1
Killed after 9 days	4
Killed before 9 days	1

Again it has to be remarked that inoculation by this method apparently failed to produce infection in only three cases out of 27 putrid rats presenting appearances strongly suggestive of plague, *i.e.* it apparently fails in 10% of putrid rats.

Some writers have stated that rats suspected of being plague-infected have come under their observation which were too putrid to permit of bacteriological examination. It would seem, however, from our experience at least, that the cutaneous method gives an excellent chance of diagnosing plague even in rats far advanced in putrefaction. In support of this statement we would refer to the account given above of an experiment in which 12 experimentally infected rats dead of plague were allowed to putrefy. Although in all these rats the putrefactive changes were very marked, yet no fewer than seven out of 12 gave plague to guinea-pigs when inoculated cutaneously.

*Infections through the skin by organisms other than plague.*

Considering the frequency with which material of all degrees of putrefaction was rubbed into the skin, it is remarkable how seldom infection by other organisms resulted. The only instances we observed are set forth in Table XXI. It is worthy of note that guinea-pigs 1, 2, and 5 continued to gain weight after the inoculation.

TABLE XXI.

Serial No.	P. M. of rat	Microscopical examination of inoculation material	P. M. of guinea-pig	Microscopical examination of guinea-pig	Cultural tests
1	Putrid; no buboes	Many putrefactive organisms, some like <i>B. pestis</i>	Killed by $\text{CHCl}_3$ 15 days after inoculation. P.M. small left inguinal gland not congested. Spleen not much enlarged, a few gray nodules	Spleen } = 0 Heart-blood }	Culture from bubo gave an organism which corresponded to <i>B. enteritidis</i> (Gaertner)
2	Very putrid. Maggoty. Many enlarged glands	Many putrefactive organisms, a few like <i>B. pestis</i>	Killed by $\text{CHCl}_3$ in 10 days. Abscess, at site of inoculation. Right inguinal bubo. Spleen decidedly enlarged. Some small nodules scattered through lungs	Nothing definite	Pure culture of cocci
3	Putrid. Congested glands in left axilla; enlarged gland in inguinal region	Numerous putrefactive organisms	Killed by $\text{CHCl}_3$ in 9 days; circumscribed abscess size of walnut at site of inoculation, no other pathological changes	Pus—a few slender bipolar bacilli. Spleen = 0	Culture on agar of pus gave growth very like plague. A smear showed cocci (?). Culture inoculated subcutaneously. Killed a guinea-pig in 2 days with a septicaemia; spleen not enlarged, but crowded with very small diplo-bacilli
4	Very putrid; small glands in axillary and inguinal region	Many putrefactive, some like <i>B. pestis</i>	Killed by $\text{CHCl}_3$ in 11 days, open sore on skin with moderate infiltration at site of inoculation. No buboes—no signs of plague. Spleen small		
5	(Live <i>decumanus</i> .) Left femoral abscess, organs congested	Doubtful	Died in 6 days. Large purulent local reaction; no buboes—in testines congested; spleen small	Organisms in spleen, not plague bacilli	

*On the comparative value of the cutaneous method in the diagnosis of rats suspected of being plague-infected.*

It would appear, from the experiment summarised in Table XVI, that when virulent plague bacilli unmixed with other organisms are inoculated into a guinea-pig, the subcutaneous method is more certain in producing infection than the cutaneous when a small dose is given. In actual practice, however, the chief difficulty arises from the admixture of other organisms in the material used for inoculation. At the commencement of our work in the routine examination of rats, we made emulsions of the organs and injected them subcutaneously into guinea-pigs. Of seven guinea-pigs thus inoculated, three died of a septicaemia due to contaminating organisms. This method had therefore to be abandoned.

With regard to the chances of success by the cutaneous method, we have already mentioned incidentally that the inoculation appeared to fail in the case of three rats only (excluding putrid rats) out of a total of 300 examined, in other words, the method failed in only 1% of the tests. If putrid rats be included, the method failed in 2% of the total experiments. In putrid rats the method appears to fail in 10% of the cases. It must be pointed out however, in qualification of this statement, that the strong suspicion of plague in these cases rested solely upon our opinion of the naked-eye and microscopical appearances of the rats, and that this in turn was the outcome of an experience in the diagnosis of plague rats from post-mortem appearances which was, at the period of examination, comparatively small.

In addition to the subcutaneous and cutaneous methods, others have been recommended, *e.g.* the conjunctival, nasal, and the "Hauttasche" methods. At present we cannot speak from personal experience of these procedures, but the ease with which the cutaneous method can be carried out would seem to give it an advantage over the others.

Pseudo-tuberculosis has never been encountered in our stock of guinea-pigs in Bombay. An epizootic of this disease, or even the occurrence of sporadic cases, would seriously complicate the use of the method for diagnostic purposes. In such an event two plans might be adopted. Either white rats might be used instead of guinea-pigs, or the guinea-pigs should be isolated in separate cages and the weight of each should be taken daily for at least a week before being used for experiment. The weighing should, of course, be continued after the test. The only disease resembling plague which has been observed by us in Bombay as a natural infection in guinea-pigs, is one caused apparently by an

organism belonging to the *B. enteritidis* (Gaertner) group. The disease chiefly affects young guinea-pigs in the monsoon months, viz. July to November. The lesions in the organs bear a remarkable resemblance to those found in plague guinea-pigs and, even on microscopical examination, the bacilli appear as bipolar organisms almost indistinguishable from *B. pestis*. Cultural tests, however, render the diagnosis a matter of no difficulty, the causative organism differing in every essential respect from the plague bacillus.

A possible limitation to the employment of the cutaneous test is the occurrence in fresh plague material of avirulent bacilli. Our experience—a not inconsiderable one—leads us to believe that this must be an exceedingly rare event, if, indeed, it ever happens. We have never isolated from an animal a strain of plague bacillus, the first remove of which can be styled “avirulent,” that is, one which fails to kill guinea-pigs by injection of massive doses. - We have worked with several such strains, but in every instance the culture has been either one isolated from an old broth culture, or one which has been frequently subcultivated outside the body. Originally these cultures were fully virulent.

#### IX. CONCLUDING REMARKS.

Although the data brought forward in this paper do not lend themselves to being condensed into the form of a summary, yet the facts which appear to us to be of principal interest may be briefly recalled.

It has been shown that plague rats, like human cases, may be divided into two classes, according as to whether or not a bubo is present. The bubo, if present, is the most important diagnostic sign of plague.

Of other characteristic appearances, those occurring in the liver of plague-infected rats have been described in detail, since they are of primary importance from the point of view of diagnosis. Haemorrhages in various parts of the body are commonly met with, and an abundant clear pleural effusion constitutes, when present, a noteworthy sign of plague in the rat.

Analyses of the results of microscopical examination of 1200 plague rats are set forth chiefly in the form of tables. It is apparent from these that the bubo gives the best chance of recognising plague bacilli in large numbers. Not only so, but the value of the bubo as an aid in the microscopical diagnosis of plague is increased by the presence in at least 50% of those examined of the characteristic involution forms.

Reference has been made to the occurrence of plague-like bacilli or of plague-like diseases in rats. We can only reiterate the statement that in Bombay no difficulty of this kind has been experienced.

The relative value for diagnosis of the macroscopical and microscopical methods of diagnosis has been discussed. The results of tests carried out for the purpose of comparison make it manifest that the naked-eye is markedly superior to the microscopical method as an aid in diagnosis, and as the result of our experience we are prepared to make a diagnosis of plague on the strength of the macroscopical appearances alone, even though the other results of cutaneous inoculation and culture are negative and the animal shows marked signs of putrefaction.

The value of the method of cutaneous inoculation of guinea-pigs has been examined: it would appear to fail only in about 2% of fresh and about 10% of putrid rats.

The bacilli found in naturally infected rats are fully virulent: 62% of the inoculated animals die of acute plague in five days or less.

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## XII. THE PATHOLOGICAL HISTOLOGY OF THE SPLEEN AND LIVER IN SPONTANEOUS RAT-PLAGUE, WITH OBSERVATIONS ON THE EXPERIMENTAL INFECTION.

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(PLATES VIII AND IX.)

The pathological histology of plague in man and experimental plague in animals has already been the subject of several researches, notably those of Aoyama (1896), Yamagiwa (1897), Albrecht and Gohn (1897), Dürck (1904) and Hamdi (1904) in the case of human plague, and of van der Stricht (1897), Honl (1897), Lustig and Zardo (1897), Babes and Livadite (1897), Albrecht and Gohn (1900) and Sata (1900) in the case of experimental plague.

The guinea-pig and rat have been mainly employed as experimental animals owing to their great susceptibility to infection, but in view of the natural occurrence of plague in the latter animal and the important part it plays in the propagation of human plague, it is highly essential that we should have all details regarding the histology of the natural rat infection even though the differences between such and the experimental form may be presumably insignificant.

The pathological histology of natural plague infection in rats has been referred to only by Albrecht and Gohn (1900) who gave brief histological details of the organs of five rats which had died of plague in Bombay. Ogata (1897) also contributed a few observations on the subject.

It is to fill up this lacuna in our knowledge of spontaneous rat plague that the following histological examinations have been made from material collected in Bombay by the Plague Commission. The primary cause, however, of this inquiry was the elucidation of the peculiar characteristic post-mortem appearances of the spleen and liver,

to which reference has been made in another article in this number. For this purpose, therefore, only the spleens and livers from the various cases were forwarded.

It was felt, moreover, that a more minute investigation of the histological changes might throw some light on the conditions leading up to the chronic form of rat plague which is associated with the presence, in the spleen particularly, of encapsulated abscesses.

To add completeness to the work and for purposes of comparison the organs of several rats which had been inoculated in Bombay with virulent plague, were submitted to detailed examination by similar methods. Finally one or two cases of chronic experimental plague in vaccinated rats were investigated histologically. The results obtained in these experimental cases will be discussed in Part II of this paper.

## PART I.

### *Spontaneous Rat Plague.*

The very brief histological details recorded by Albrecht and Gohn may be here summarised:—

Rat I. *Spleen*:—Numerous haemorrhages were present in the pulp. Necroses had also commenced especially at the periphery of the nodes. Bacilli were scarce. *Liver*:—Showed cloudy swelling. Bacilli scarce.

Rat II. *Spleen*:—Pulp was haemorrhagic and infiltrated with polynuclear leucocytes. The nodes were surrounded by a fine or coarse network of connective tissue. Bacilli were numerous. *Liver*:—Liver cells showed karyorrhectic nuclei in many cases. The capillaries contained enormous masses of bacilli.

Rat III. *Spleen*:—Pulp contained large amounts of nuclear debris especially in the vicinity of the nodes. *Liver*:—Showed degeneration of parenchyma. Capillaries were full of bacilli.

Rat IV. *Spleen*:—Pulp presented commencing necroses. Bacilli numerous.

Rat V. *Spleen*:—Numerous haemorrhages and necroses in the pulp. The peripheral portions of the nodes and the adjoining pulp-tissue were transformed into a coarse network staining intensely with eosin.

Rat VI. *Spleen*:—Showed haemorrhages and nuclear disintegration. Bacilli very numerous. *Liver*:—Liver cells showed fatty degeneration. Bacilli very abundant in the capillaries.

Ogata (1897), during the epidemic of plague in Formosa, obtained six rats which had died of plague. Referring to the condition of the organs he merely remarks that the spleen was much swollen and the liver congested. Small haemorrhages were also present in the liver and bacilli were numerous, as also in the spleen and glands.

From the above summary, it will be evident that the grosser lesions only have been recorded, possibly owing to the difficulty of procuring material fresh enough for detailed histological examination. The organs at my disposal were the spleens and livers of thirteen cases of rat plague. These had been fixed in Orth's fluid and sent to this country in spirit. The tissues were embedded in paraffin and the stains chiefly employed were Unna-Pappenheim's methyl-green-pyronin and Ehrlich's haematoxylin and orange-rubin. The former stain proved eminently satisfactory for the demonstration of plague bacilli and plasma cells.

Rat I. *Protocol* :—Primary bubo—left axillary. Congestion of spleen and subcutaneous tissues. Bacilli present in bubo, heart blood and spleen.

*Spleen* :—Capsule : shows no pathological changes.—Nodes : The nodes are few in number but regular in outline. Karyorrhexis of the lymphoid cells of the node is a marked feature and a large amount of nuclear detritus is lying either free or included in large endothelioid phagocytic cells. Some of these particles show their cytoplasmic origin by taking up the pyronin stain. Large mononuclear cells of endothelioid type are abundant throughout the node, many of them presenting mitotic figures (see Plate VIII, Fig. 2). The protoplasm of these cells stains a deep red, the nucleus being rather vesicular. No bacilli were detected within the node though a specially rich zone of them was present in the perinodal lymph sinus.—Pulp : Extravasation of red cells was very marked and plague bacilli were fairly uniformly distributed and in large numbers. No necrotic foci were present. Rows of plasma cells, many showing mitosis, were arranged in the sheaths of the trabecular vessels. *Liver* :—The protoplasm of the liver cells was coarsely vacuolated. Necrotic foci, which are the outstanding feature of plague livers, were in this case few in number and of very small size, in some cases only two or three liver cells being involved. Within these necrotic areas were a few vesicular nuclei and occasionally a small group of bacilli lying in what remained of the intraacinar capillaries. Enormous numbers of bacilli were present in the liver capillaries. Many of the liver cell-nuclei showed karyorrhexis.

Rat II. *Protocol* :—Primary bubo—left submaxillary. Spleen, lungs, and subcutaneous tissues congested. The surface of the liver had a mottled appearance. Bacilli in spleen and bubo.

*Spleen* :—Subcapsular haemorrhage was considerable.—Nodes : These were greatly diminished in number. Large mononuclear endothelioid cells were numerous, the small lymphoid cells showing extreme karyorrhexis. In some nodes were small necrotic foci containing large endothelioid cells and nuclear detritus, similar to those described in the diphtheritic spleen (see Waschewitsch *Virch. Archiv*, Bd. 159, 1900).—Pulp : There was great congestion of the pulp sinuses accompanied with red cell extravasation. Around the trabecular vessels were many plasma cells showing extreme karyorrhexis and pyknosis (see Plate VIII, Fig. 5). The cells of the spleen pulp presented as a whole only a slight degree of karyorrhexis and no actual necrotic foci were observed. Bacilli occurred in swarms throughout the pulp especially in the areas of blood extravasation. *Liver* :—The protoplasm of the

## EXPLANATION OF PLATES VIII AND IX.

**Plate VIII.** Fig. 1. Portion of necrotic focus of liver (natural rat plague). *a*=Blood in capillaries. *b*=Remains of liver-acini. *c*=Liver cell showing vacuolar honeycomb degeneration. *d*=Mononuclear cell in capillary.

Fig. 2. Portion of malpighian body (natural rat plague) showing large mononuclear endothelioid cells. *a*=Mitosis of the same. A degenerated endothelial cell containing nuclear detritus is also seen.

Fig. 3. Portion of spleen in chronic experimental rat plague. *a*=Giant-cell (of tubercular type). *g*=Commencing agglomeration of nuclei to form giant-cell. *b*=Megakaryocyte. *d*=Granulation spindle cell. *c*=Rows of plasma cells. *f*=Leucocytic debris round bacilli. *e*=Degenerated bacilli.

Fig. 4. Portion of liver (natural rat plague). *a*=Megakaryocyte in capillary. *b*=Liver-acinus.

Fig. 5. Portion of spleen-pulp (natural rat plague) showing bacilli and pyknotic plasma cells of all types, and effused red cells.

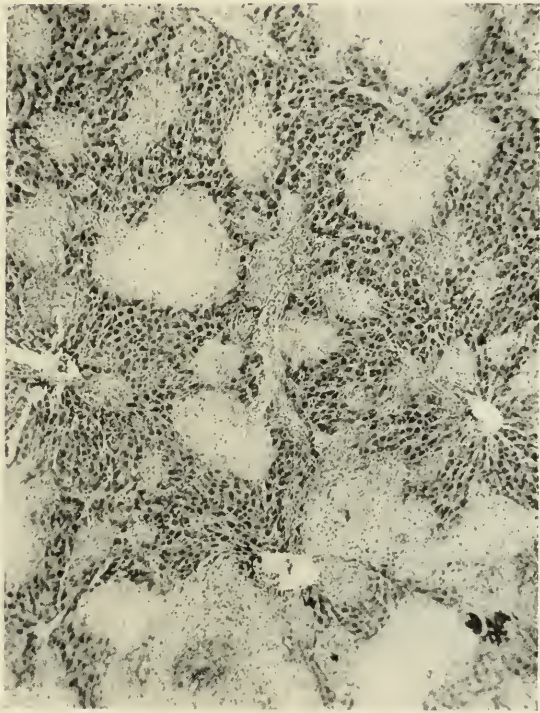
Fig. 6. Giant-cell (of Langhans' type) from periphery of malpighian body (experimental rat-plague). The drawings are made from five successive sections through the same cell. Note the grape-like nuclear agglomeration in the 4th drawing.

**Plate IX.** Photomicrograph of liver necroses (natural rat-plague).











liver cells was finely vacuolated but not fatty. Acinar necroses were few in number and of very irregular contour. In the capillaries of the necrotic area, bacilli were still present. The portal vessels and intraacinar capillaries were extremely congested and contained large numbers of bacilli.

Rat III. *Protocol* :—Primary bubo—right submaxillary. The liver had a granular appearance and presented minute haemorrhages on the serosa. Bacilli present in heart blood, spleen and bubo.

*Spleen* :—The capsule presented no marked changes.—Nodes : These were few and of irregular shape and size and showed the characteristic endothelioid proliferation with numerous mitoses. At the periphery of the node plasma cells were abundant. A marked feature was the presence of a reticular zone encircling each node. The few pulp cells remaining in this zone showed extreme karyolysis and bacilli were specially abundant.—Pulp : The pulp showed great congestion with haemorrhages and polynuclear infiltration. No definite necrotic foci were seen although bacilli occurred in swarms. Plasma cells were numerous in the sheaths of the vessels and there was a very pronounced catarrh of the vascular endothelial cells. *Liver* :—Focal liver cell necroses were fairly numerous especially in the subcapsular area. They were apparently of recent origin and contained many extravasated red blood corpuscles with bacilli still lying in the capillaries. The liver cells showed fine vacuolation of their cytoplasm. Bacilli were not quite so numerous in the vascular system of the liver as in the previous cases.

Rat IV. *Protocol* :—Primary bubo—right submaxillary. The serosa of spleen and liver had a granular appearance. Bacilli few in heart blood, spleen and bubo.

*Spleen* :—The capsule was markedly thickened and oedematous, the subcapsular lymph space showing extreme endothelial catarrh.—Nodes : The nodes were greatly diminished in number and in some cases contained rounded areas with vacuolated endothelial cells and nuclear debris. Many lymphoid cells had undergone karyorrhexis.—Pulp : Even with low magnification large clumps of bacilli could be seen throughout the pulp, each surrounded by a zone of karyorrhectic nuclei. The condition was thus one of multiple small abscess formation. Around the nodes and vessel-sheaths plasma cells and vesicular epithelioid cells abounded. The karyorrhectic focus was immediately surrounded by a zone of vesicular epithelioid nuclei and this again by a barrier of plasma cells. These epithelioid cells showed a slight tendency to nuclear grouping but no fully formed giant-cells of tubercular type were met with. *Liver* :—Liver cell necroses were present in enormous numbers and in fact little healthy liver tissue was left (see Plate VIII, Fig. 1 and Plate IX). Some foci contained only red blood corpuscles, with no traces of bacilli remaining, while others contained a central clump of bacilli surrounded by karyorrhectic nuclei or proliferating epithelioid cells. The production of some necroses by vascular bacillary emboli was quite patent though in others such a connexion could not be traced. Among the vesicular nuclei surrounding one focus a fully formed giant-cell of tubercular type was detected. In the healthy liver capillaries, bacilli were scarce and evidently degenerated, their remains being taken up by the endothelial cells of the vessel wall. A very interesting feature was the presence in the dilated capillaries of great numbers of epithelioid cells some of which were undergoing mitosis. An occasional giant-cell of the splenic megakaryocyte type was detected in the capillary lumen. Round the portal vessels in Glisson's capsule small lymphomata were sometimes met with.

Rat V. *Protocol* :—Congestion of subcutaneous tissue, lungs and spleen.—Liver fatty and granular. No bacilli in heart blood, spleen or liver.

*Spleen* :—The capsule is very oedematous.—Nodes : There is no diminution in the number of malpighian bodies, each containing many endothelioid cells with plasma cells at the periphery. Among the latter, very many mitotic forms were observed. A perinodal zone of reticular tissue with few nuclei was a constant feature, forming a species of capsule for the node.—Pulp : The pulp was extremely congested but only a few bacilli were detected in small groups lying among the extravasated red blood corpuscles. Many necrotic foci were present. These at times consisted of karyorrhctic nuclei and detritus or of vesicular epithelioid cells. The plasma cell reaction was very conspicuous. Pigment cells containing haemosiderin and catarrhal endothelial cells occurred in fairly large numbers. *Liver* :—Very numerous sharply demarcated necroses were present in all stages of development. In some of them, red blood corpuscles were the predominant elements, while in others the endothelial cells of the capillaries showed a marked proliferation. A few bacilli could still be detected in these foci. Elsewhere, the capillaries of the liver contained here and there small bunches of bacilli which appeared to have undergone degenerative changes. The phagocytic endothelial cells frequently contained such bacillary debris.

Rat VI. *Protocol* :—Double pelvic bubo containing swarms of bacilli. No bacilli in heart blood or spleen.—Liver coarsely granular and spleen congested.

*Spleen* :—The capsule was oedematous and the subcapsular area very much congested, the endothelial cells actively proliferating.—Nodes : These were much diminished in number but presented only slight pathological changes.—Pulp : The pulp was greatly congested. There were no definite areas showing karyorrhesis of the pulp cells but here and there could be seen foci in which the nuclei had disappeared or had become vesicular. Pyknotic plasma cells were present in moderate numbers. No bacilli could be demonstrated. *Liver* :—The liver cells looked fairly healthy. Focal necroses were moderate in number, a few remains of bacilli being still apparent in them. The portal vessels were extremely congested. Free bacilli were never seen in the capillaries but the endothelial cells contained here and there what appeared to be bacillary remains.

Rat VII. *Protocol* :—Primary bubo unknown. Liver fatty and granular.—Spleen granular. Many bacilli in spleen.

*Spleen* :—The capsule was oedematous and infiltrated with red cells, the superficial endothelium being catarrhal.—Nodes : These showed no important changes. No bacilli were detected in them.—Pulp : Large irregular necrotic areas were present showing no very definite boundary and frequently lying alongside a malpighian body. At the margins of these areas or inside them were wisps of bacilli. In one case, a vein leading to such a focus was completely blocked by plague bacilli for a considerable distance. Extraordinary numbers of bacilli were present in the pulp among the extravasated red cells and polynuclear infiltrates. Karyorrhctic and pyknotic plasma cells and catarrhal endothelial cells were specially numerous. *Liver* :—The liver cells throughout the lobule showed large protoplasmic vacuoles due to fat. Focal liver cell necroses were very numerous and sharply demarcated from the healthy liver substance. Some foci contained very few nuclei, while others contained numerous vesicular nuclei tending at times to coalesce. The contents of



the capillaries were polynuclear cells, red cells and occasional degenerated bacilli. A few small lymphoid nodes were observed around the portal vessels in the large trabeculae. Large mononuclear endothelioid cells showing mitoses could be seen in these small lymphomata as in the splenic nodes.

Rat VIII. *Protocol* :—Primary bubo—left axillary. Liver fatty and granular.—Spleen faintly granular.—No bacilli in heart blood or spleen.

*Spleen* :—The chief feature was the presence of numerous pulp necroses in which only a few vesicular nuclei remained. These areas were invariably perinodal. Outside these areas the pulp cells showed slight karyorrhexis. Pyknotic plasma cells occurred in enormous numbers. Bacilli were exceedingly scarce and confined to the necrotic foci. *Liver* :—Cell necroses were very numerous and contained nuclear debris and vesicular epithelioid cells. Sometimes a few degenerated bacilli could be seen in them. Elsewhere, the liver capillaries contained numerous degenerated endothelial cells and karyorrhectic nuclei. Bacilli were very scarce and only in phagocytic endothelial cells. There was marked congestion throughout.

Rat IX. *Protocol* :—Primary bubo—left submaxillary. Liver fatty and granular.—Spleen granular.—No bacilli in heart blood, spleen or bubo.

*Spleen* :—In this case the areas of pulp degeneration were very numerous and extensive. The malpighian bodies were encroached upon and very little normal splenic tissue remained. In the degenerated areas were enormous masses of nuclear detritus with occasional degenerated bacillary clumps in the centre. Bounding these areas were rows of plasma cells in active division and catarrhal endothelial cells. Signs were already present of the replacement of these foci by spindle-shaped granulation cells formed from the actively dividing plasma cells. *Liver* :—Necroses were few but of all sizes. Some bacilli still remained in the capillaries of the necrotic area along with red cells. Throughout the liver substance, the capillaries were greatly dilated with red cells, desquamated endothelial cells containing nuclear detritus and large mononuclear cells suggesting a splenic origin. Typical giant cells of megakaryocyte type were also met with not infrequently lying in the capillary lumen (see Plate VIII, Fig. 4). It seems most probable that these cells, along with many of the types filling up the liver capillaries, have found their way to this organ from the spleen. Babes and Livadite (1897) noted the presence of similar giant cells in the guinea-pig's liver (in experimental plague), but apparently assigned to them a different origin. They write "Sehr bemerkenswerth ist das Auftreten von Riesenzellen mit gelappten Kern wohl auf Kosten gewisser Endothelien." Only a very few bacilli were present in the capillaries.

Rat X. *Protocol* :—No primary bubo. Liver fatty and granular.—Spleen congested.—No bacilli in heart blood, spleen or liver.

*Spleen* :—Not available for histological examination. *Liver* :—Liver cell necroses were few and of small size. The subcapsular region was mainly the site of these foci and there also bacilli were most frequent. The capillaries of the necrotic area were blocked by bacilli in many cases. Throughout the liver substance the capillaries were dilated with red cells and splenic elements as in the previous case. Megakaryocytes were also occasionally detected. Bacilli as a rule were scarce and many were included in the endothelial phagocytes.

Rat XI. *Protocol* :—Primary bubo—left axillary. Liver and spleen congested. Many bacilli in spleen and bubo.

*Spleen* :—The lymphoid tissue was greatly diminished. Large mononuclear endothelioid cells in active division were present in the nodes. Necrotic areas containing vesicular nuclei and bacillary debris were distributed throughout the pulp. Megakaryocytes, pyknotic plasma cells and catarrhal endothelial cells were specially abundant. An interesting feature was the presence of a large number of eosinophile cells. *Liver* :—Necroses were few but of all sizes. The capillaries were dilated as in the previous cases with splenic elements. Plasma cells were also numerous. A slight degree of perivascular lymphoid infiltration was noted in the portal spaces.

Rat XII. *Protocol* :—Liver coarsely granular and fatty, spleen finely granular.—Subcutaneous and pulmonary haemorrhages.—No bacilli in heart blood, few clumps in spleen.

*Spleen* :—The nodes were few in number and badly differentiated from the surrounding pulp.—Pulp : Small irregular necrotic foci were distributed throughout the pulp. The centre of each focus was occupied by swarms of bacilli and the periphery by vesicular nuclei, detritus and blood-pigment-carrying cells. The subcapsular area was infiltrated with red cells and polynuclear leucocytes. Megakaryocytes were exceedingly numerous especially in the neighbourhood of the necrotic foci. *Liver* :—Fatty infiltration of the liver cell-protoplasm was far advanced. Many nuclei also exhibited karyorrhexis. Only a few small necrotic foci were noted. The intraacinar capillaries were greatly dilated, their lumina being filled with small and large mononuclear cells and endothelial phagocytes.

Rat XIII. *Protocol* :—Lungs both consolidated. Gray hepatisation of upper and middle lobes of right lung.—Liver has a mottled appearance.—Spleen congested.—Swarms of bacilli in heart blood, spleen and bubo.

*Spleen* :—Malpighian bodies were numerous and of irregular form and size. Many karyorrhectic nuclei were present in the nodes along with large endothelial cells containing nuclear detritus.—Pulp : A few clumps of degenerated bacilli were seen here and there but no necrotic foci were in evidence. The cell types met with in the pulp were very varied, small and large mononuclear cells, endothelial cells, megakaryocytes and nucleated red cells. An interesting feature was the great abundance of coarsely granular eosinophile cells many of which showed mitotic figures. They occurred in greatest numbers in the subcapsular area. A few clumps of mast cells were also noted. Plasma cells were very scarce. In this case the pneumonia was evidently the main plague-lesion, while the spleen presented none of the characteristic changes observed in the foregoing cases. The splenic picture, in fact, was that associated with an actively functioning haemopoietic organ. *Liver* :—Unsuitable for histological examination.

### *Survey of the above cases.*

The cases may be divided into two main groups :—

1. Those in which bacteriaemia of the spleen and liver is at a maximum and has been of recent development.
2. Those in which bacteriaemia is less prominent or is rapidly disappearing as a result of reactive tissue changes.

In the first group, the incursion of bacillary swarms into the spleen and liver has been accompanied by extensive haemorrhages and congestion of the pulp sinuses and liver capillaries. Definite abscess formation in the spleen has not had time to develop and focal liver cell-necroses are few in number, the latter being very largely confined to the sub-capsular region.

In the second group, definite abscess formation in the spleen is far more frequent and is accompanied by extensive reactive changes on the part of the plasma cells. The reduction in the amount of lymphoid tissue is due in great measure to the perinodal distribution of degenerated pulp areas. A barricade of plasma cells separates the lymphoid tissue of the node from the necrotic zone. Proliferation of the large mononuclear endothelioid cells of the node is a noteworthy feature. Dürck (1904) has described a similar change in the splenic nodes in human plague.

In the liver, focal necroses may be so numerous that little healthy liver tissue remains. The demonstration of bacilli in the central capillaries of these foci was made in nearly every case and frequently actual bacillary embolism was noted. The formation of giant-cells of Langhans' type in the neighbourhood of necrotic foci is also of great importance, although in some of the cases these cells had not reached their full development. It can readily be conceived how, providing the animal lives long enough, the reaction of the fixed tissue cells may proceed to complete encapsulation of abscess areas and so bring about a more or less chronic condition. So far I have had no opportunity of examining histologically such cases of chronic spontaneous rat plague, but it is hoped that suitable material of this kind may soon be available. The chronic experimental case, which will be described later, gives however a very clear notion of the later stages in this process of abscess-encapsulation.

Frequent mention has been made in the protocols of a granular and mottled appearance of the liver. The spleen has also been described as granular in some cases. In the case of the liver such changes are readily accounted for by the distribution of the haemorrhages and the focal necroses together with the fatty changes in the liver cells. It must be understood, however, that a peculiar, honeycomb-like vacuolar degeneration of the liver cell protoplasm was far more frequent than an actual coarse fatty infiltration. The granular appearance of the spleen is due partly to endothelial catarrh and partly to subcapsular changes.

With regard to the disappearance of bacilli from the intraacinar capillaries of the liver it appears that phagocytosis by endothelial cells is largely responsible. Indeed in some cases no free bacilli were demonstrable. The presence of large giant-cells of megakaryocyte type in the liver capillaries of some cases is highly interesting: in such cases the capillaries generally contained so many extraneous cell elements that one is forced to assign to them a splenic origin.

Finally, with regard to the distribution of plague bacilli in the spleen, it was exceedingly rare to find the organisms in the interior of the nodes. Yet, though bacilli were absent, karyorrhexis of the nodal cells was frequently far advanced. Toxaemia must then be largely responsible for the alterations in the nodes, as we know that analogous changes take place in the malpighian bodies after the inoculation, for instance, of diphtheria toxin.

## PART II.

### *Experimental Rat Plague.*

The following is a brief *résumé* of our knowledge regarding the histology of the spleen and liver in experimental plague.

*Spleen.* In the guinea-pig van der Stricht (1897) noted a diminution in the size of the malpighian bodies with dilatation of the capillaries of the node, leading to actual rupture. Abscesses occurred in the pulp but did not affect the nodes. The splenic megakaryocytes were very numerous and the capsule of the organ was infiltrated with white corpuscles. Honl (1897), working with the same animal, found an enlargement of the follicles and extreme congestion of the pulp. Bacilli occurred in "zoogloal" groups round which were numerous fragmented cell elements. Lustig and Zardo (1897) working with rats, mice, guinea-pigs and rabbits noted that the periphery of the follicle was the seat par excellence of the necrotic foci. The trabeculae had a hyaline appearance and the arteries were dilated. Haemorrhage into the pulp was a constant feature. Plague bacilli were numerous throughout the pulp and might occur inside the nodes. Babes and Livadite (1897) recorded in guinea-pigs and mice the presence of large numbers of giant-cells of megakaryocyte type, especially in the vicinity of the necrotic areas. Blood-corpuscle-containing cells and pigment cells were scarce in spite of extensive pulp haemorrhages.

Albrecht and Gohn (1900) found a great similarity in the main



splenic lesions in experimental rat plague by whatever method the inoculation was performed. The nodes were as a rule much diminished and necrotic pulp-foci were of constant occurrence.

Sata (1900) gives a fairly detailed description of the histology of experimental rat plague and lays great stress on the variations met with as regards the distribution of plague bacilli in the organs. Subcapsular haemorrhage was a frequent feature in the spleen and bacilli might be very numerous, very scarce or not demonstrable at all in sections.

*Liver.* Van der Stricht observed areas of fatty degeneration of the liver cells and also a fine vacuolated condition of the liver cell protoplasm. In one animal small necrotic areas due to capillary emboli were noted.

Honl noted focal necroses surrounded by a zone of leucocytes. Groups of bacilli arranged in zoogloal masses were present in these foci. Babes and Livadite recorded the presence in the liver capillaries of "Riesenzellen mit gelappten Kern" which have been already alluded to.

Fatty degeneration of the liver cells was noted both by Albrecht and Gohn and Sata. The latter also demonstrated fibrin in the larger vessels. The occurrence of bacilli in the liver capillaries was found to be a very variable factor.

The material of the following six cases was obtained from rats inoculated by the cutaneous method in Bombay. For Rat No. VII of chronic experimental plague I am indebted to Capt. S. R. Douglas.

Rat I. *Spleen* :—Serosus endothelium swollen, capsule oedematous with effusion of red cells into it. The endothelial cells of serosa contained red cell debris. There was marked subcapsular haemorrhage.—Nodes : The lymphoid tissue was increased in amount. At the periphery of each node was a circular zone of necrotic cells with bacillary clumps in the neighbourhood. Near the margin of one node was a typical Langhans' giant-cell. The appearance of this cell in five serial sections is indicated in Plate VIII, Fig. 6. In one cross section the constituent nuclei are seen to fill practically the whole cell leaving only a faint rim of protoplasm. The pulp was much congested and numerous zoogloal bacillary masses were noted. Plasma cells were abundant. The pulp cells showed a slight degree of karyorrhexis. No bacilli were seen in the nodes. *Liver* :—The liver cells were generally healthy apart from the necrotic areas which were numerous. Each focus contained small heaps of bacilli and a good deal of haemorrhage was present at the periphery. Endothelial catarrh of the capillaries was a marked feature. Bacilli were distributed in clumps and many of them were noted inside phagocytic endothelial cells. Fibrin was also observed in the capillaries, along with numerous red cells.

Rat II. *Spleen* :—Marked catarrh of the serous endothelium with subcapsular haemorrhage. The lymphoid tissue was increased and irregularly distributed. Numerous actively dividing large mononuclear endothelial cells were observed in the



nodes. A zone of karyorrhectic pulp cells with detritus forms a sort of capsule to each node. Bacilli occurred in clumps surrounded occasionally by necrotic pulp cells. Pyknotic plasma cells were very abundant. In the haemorrhagic areas, fibrin was present in large amount and blood-corpuscle-containing cells were numerous. *Liver*:—Liver cells were not fatty. Marked subcapsular haemorrhage. Necroses were few and small and most of them contained bacillary clumps, surrounded by karyorrhectic nuclei. Bacilli were very numerous in the capillaries and the endothelial cells frequently contained ingested bacilli.

Rat III. *Spleen*:—Capsule swollen and subcapsular haemorrhage. The malpighian bodies were swollen but regular in contour. Round each was a zone of thickened reticulum containing few cells. For the first time, a node was seen whose central artery contained a fibrin thrombus with bacilli. In the pulp, enormous numbers of bacilli were present along with red cells and fibrin. Polynuclear cells, pyknotic plasma cells and endothelial cells were abundant. Haemosiderin cells also occurred frequently. *Liver*:—The liver cells were markedly fatty. Their nuclei also frequently presented karyorrhexis. Necroses were fairly numerous and often of large size. Capillaries containing bacillary emboli were noted in these foci. In the capillaries of the healthy liver substance were enormous numbers of bacilli generally clinging to the walls of the vessels. Round the portal vessels were lymphoid and plasma cell infiltration.

Rat IV. *Spleen*:—Large numbers of bacilli surrounded each node but in only one case were bacilli seen in the follicular artery. The large mononuclear endothelioid cells were abundant in the node and showed signs of active division. Also small necrotic areas containing large endothelial phagocytes and detritus sometimes appeared inside the node. The pulp showed extensive haemorrhages with polynuclear infiltrates and haemosiderin cells. No necrotic foci were present but here and there were small areas in which the cell-nuclei were vesicular. *Liver*:—The liver cells were fatty. Only one or two minute necroses were seen each containing bacilli in its central capillary with effusion of red cells at the periphery. The capillaries throughout the liver substance were quite filled with bacilli and many polynuclear cells were present. Colonies of plasma cells were seen in the sheaths of the large portal vessels in the Glisson's space.

Rat V. *Spleen*:—Capsule thickened, trabeculae increased, and marked subcapsular haemorrhage. The nodes were greatly diminished and irregular in contour. They contained numerous large mononuclear cells of endothelioid type, many of which were dividing. The spleen pulp was greatly congested and bacilli occurred in swarms especially in the large trabecular sinuses. Polynuclear cells, haemosiderin pigment cells and blood-corpuscle-containing cells were abundant in the areas of red cell extravasation. Plasma cells were very numerous and were in active division. No actual pulp necroses were observed. *Liver*:—Only a very few minute necrotic foci were observed in the subcapsular region, involving individual liver cells or a few adjacent ones. Bacilli were also most numerous in this region, lying in clumps in the capillary vessels. The intraacinar capillaries throughout the organ were filled with red cells, infiltrating cells of polynuclear type, and catarrhal endothelial cells.

Rat VI. *Spleen*:—Marked subcapsular haemorrhage and great reduction in the amount of lymphoid tissue. Many of the nodes showed a hyaline thrombosis of the central artery, the lymphoid cells in the neighbourhood being karyorrhectic.

Many endothelial phagocytic cells were present, containing nuclear detritus in their interior. A perinodal zone of karyorrhectic pulp cells was a conspicuous feature. Vesicular epithelioid cells and plasma cells invariably bounded this zone. The pulp was greatly congested with extravasated red cells and polynuclear leucocytes. Bacilli occurred in enormous numbers. Haemosiderin cells and megakaryocytes were also very abundant. *Liver* :—Necroses were few in number. Bacilli were generally to be found in the central capillaries of the focus with effused red cells at the periphery. The large portal sinuses and the intraacinar capillaries throughout the organ were extremely congested and contained enormous numbers of bacilli and polynuclear leucocytes.

Rat VII. *Chronic experimental rat plague.*

The rats in which these chronic plague lesions were found had been inoculated with virulent plague ten days after a partial immunisation with plague vaccine. Those which survived were killed on the eleventh day following the inoculation with the virulent culture.

*Protocol* :—Spleen much enlarged and contained large grayish caseous-looking areas.—Liver also showed a few grayish nodules.

*Spleen* :—Microscopical examination (Unna-Pappenheim's stain). No differentiation of the splenic tissue into nodes and pulp was possible. In fact the organ was transformed into a veritable plasma-cell granuloma with abscesses interspersed here and there. A large clump of degenerated bacilli occupied the centre of each necrotic area and all around were broken down polynuclear cells. Bounding this zone of degenerated cells was a band of epithelioid cells, spindle cells and numerous giant-cells of tubercular type. Megakaryocytes also appeared in this zone. Enclosing the whole was a barricade of plasma cells in active division, and transition forms were readily demonstrable between these latter cells and the spindle cells from which the granulation zone surrounding the abscess was being developed (see Plate VIII, Fig. 3). The presence of giant-cells of Langhans' type was especially interesting as confirming the view that the small agglomerations of nuclei above referred to in some of the cases of spontaneous plague were really developing giant-cells. Fully formed cells of this type have been already noted in the liver of Rat IV, Part I. *Liver* :—A section through one of the small subcapsular nodules showed that nothing remained of the original abscess. The nodule consisted solely of spindle cells and fine connective tissue fibres with a boundary zone of actively proliferating plasma cells.

#### *Survey of the experimental cases.*

The changes met with in the experimental cases present far more points of resemblance with those of group I of the spontaneous cases than with those of group II.

Extreme bacteraemia is the rule and the infiltrating cells are found to belong mainly to the polynuclear type. In the spontaneous cases the latter feature was not so prominent.

Focal necroses of the liver cells were invariably scanty. The vessels were all greatly congested and contained large deposits of fibrin.

In the spleen, a noteworthy feature in two cases was the occurrence of thrombosis of the follicular artery accompanied by bacillary incursion and the production of karyorrhectic changes in the nodal cells. Giant-cell formation (of Langhans' type) had evidently not proceeded to any extent, but the discovery of a typical cell of this nature at the periphery of one follicle and bordering a necrotic patch shows that, even in acute experimental cases, the tendency to giant-cell development is at least not in entire abeyance.

The importance of Rat VII, as showing the later stages of the pathological processes already at work in the more acute cases, has been sufficiently alluded to at the close of the histological summary.

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### XIII. TRANSMISSION OF PLAGUE BY FEEDING RATS WITH INFECTED MATERIAL.

#### *Introduction.*

During the last ten years, in which plague has been experimentally investigated, much work has been done with the view of settling the questions (1) as to whether it was possible to produce the disease in animals by feeding, and (2) as to whether this method of transmission played an important part in the epizootic or epidemic spread of the disease.

The Indian Plague Commission (1901) pointed out that while three workers in India, namely Gibson, Hankin and Simond, had failed to infect rats by means of feeding, both the German and Austrian Plague Commissions had been able to give the disease to animals by this method. They concluded that, while it was possible to produce plague by feeding, this method was probably not a common one of infection in nature. The German Plague Commission (1899) found that plague could be conveyed to rats by feeding them on plague cultures or on the bodies of rats dead of plague. They thought that in a state of nature rats generally became infected in this way, and that the infective material might penetrate into the system through the mucous membrane of the throat or nose and not through the mucous membrane of the alimentary canal. The Austrian Plague Commission (1898), experimenting with gray rats, also found that infection could easily be given *per os*. They never saw any post-mortem appearances to justify an inference that the primary infection had been through the mucous membrane of the stomach. If large amounts of material were used, or if the stomach tube was employed, then intestinal plague was produced. They succeeded, however, in producing in various animals a primary neck bubo, pointing to the entrance of the plague bacillus through the mucous membrane of the mouth,



Kolle (1901) fed 60 white rats partly with bread soaked in cultures and partly with the organs of plague-infected rats. Of 48 successful infections, 40 had primary buboes in the submaxillary region, four showed a plague pneumonia, two had mesenteric buboes, and two showed foci of infection in the small intestine. The intestinal lesions fell principally on Peyer's patches, which were enlarged, haemorrhagic and frequently showed a necrotic centre. In consequence of these observations Kolle formed the opinion that plague spread naturally among rats by their gnawing the corpses of their dead plague-infected comrades.

Kister and Schumacher (1905) did a large number of feeding experiments. They found that only 50% of rats fed developed the disease: that if spicules of bone were present in the feeding material the bubo was cervical, but that if soft food was given, intestinal plague resulted. The infected rats died as a rule on the 3rd to 5th day although some lived as long as the 8th, 9th, or 11th day. They attempted to do a series of passages by this method of infection, but failed after the third passage. They thought such a failure might explain the diminution of the natural epizootic. These workers further showed that the natural resistance of rats seemed to be heightened by several feedings with plague material, as a considerable number of animals which had been fed with large amounts over a long period of time were resistant to large subcutaneous injections of virulent culture.

Berestneff (1906) fed white rats on the livers and spleens of septicæmic plague-infected guinea-pigs. There were 12 successful infections, nine rats dying between the 3rd and 6th days. One had a bubo in the neck, another in the inguinal region, while the remaining ten showed the intestinal form of plague. In all these cases mesenteric buboes were present, while they all showed multiple lesions of the lymphatic apparatus of the small intestine. Peyer's patches were swollen and showed as prominent swellings from outside: there were punctiform haemorrhages on the serous surfaces, while the mucous surfaces were inflamed and haemorrhagic. In the caecum of one animal which died on the 7th day there were ulcers with infiltrated and haemorrhagic edges, the mesenteric glands being enlarged and haemorrhagic.

Klein (1906), who had previously failed (with rare exceptions) to infect animals by feeding with fresh plague material, has recently succeeded in giving the disease to rats, mice and guinea-pigs by feeding them with dried gelatine cultures and with dried organs of



plague-infected animals, either alone or mixed with rice and wheat. In every instance there were present enlargement and congestion of the mesenteric glands, with definite pathological changes in the intestines, the lesions being especially marked in and around Peyer's patches. The infected animals died between the 4th and 6th day.

*Methods of observation.*

In our present observations we have fed a large number of wild rats with plague-infected material. With the exception of the Punjab experiments, no distinction was made between the species of rat used, but, as a matter of fact, the great majority were *Mus rattus*. In the first and much the larger series the rats were procured from the Health Department of the Municipality of Bombay. They had been caught in traps placed in houses and gullies in the city. Before being used for our experiments they were kept isolated for some time in order to make sure that they were not already plague-infected. With a view of testing the relative susceptibility of wild Bombay rats to plague-infection by feeding in comparison with other wild rats, we did a second small series of experiments, using for this purpose rats which were caught on board ships in the harbour of Bombay and which had presumably not been subjected to an epizootic of plague.

The material used for feeding in these two series was the same. The internal organs of a rat or a guinea-pig, dead of acute plague, were minced up into more or less of a pulp. This pulp was then mixed thoroughly with the food, which consisted entirely of parched Indian corn, known in India as "laya." About 1/6 or 1/10 part of the internal organs of one animal, in which were included lungs, heart, liver, spleen, kidneys and sometimes intestines, was used for each feeding. Care was taken that no spicules of bone were present. Each rat only received one meal of this plague-infected material, so that the period of death after infection could be accurately determined.

Cultures were taken and animal experiments made from all those rats which showed any post-mortem appearances, however slight, pointing to their being plague-infected. It was possible, in this way, accurately to separate those rats which died from plague from those which died from some other cause. All rats which still survived at the end of three weeks were killed and examined post-mortem. This examination revealed the fact that a few animals, which to all appearances were well and healthy, were really plague-infected. A reference will be made to this group later on.

A third series of experiments was made in the Punjab. As *Mus decumanus* is not found in the villages of this Province, *Mus rattus* was used throughout. The material employed was the livers and spleens of rats and guinea-pigs which had been artificially infected with plague or the same organs of plague-infected rats found dead in the villages. Only one feeding was given, the amount of material being as a rule half a liver and spleen to each animal, but in the case when the infected rat weighed under 100 grammes the whole liver and spleen were used. The amount of material given to these rats was, therefore, considerably greater than in the case of the Bombay rats.

The material used for feeding in all three series of observations was soft material mixed with the ordinary food of the rats. In order to get over a possible objection that feeding with such material might cause different post-mortem lesions or a different distribution of the primary bubo to feeding with material containing bone, such as would occur in nature, a fourth series of experiments was carried out. In this series wild rats caught in the city of Bombay (no distinction being made between *M. rattus* and *M. decumanus*) were kept without food for 24 hours. They were then supplied with the carcasses of fresh plague-infected rats which had been found dead in the city, one carcass being given to every two rats. A partial post-mortem examination of each carcass, sufficient for the diagnosis of plague, had been made beforehand. In this way the conditions obtaining in nature were imitated as far as was possible.

### Results.

In Table I are summarised the general results of the experiments.

TABLE I.

Series	No. of rats observed throughout	Number which died of plague on each day								No. found plague infected when killed on 21st day	Percentage of rats which became plague infected
		2nd	3rd	4th	5th	6th	7th	8th-21st	Total		
I. Bombay rats	415	1	25	26	14	9	4	4	83	6	21.4
II. Ship rats	41	1	4	6	3	1	1	1	17	1	43.9
III. Punjab rats	28	3	11	4	1	0	0	0	19	0	67.9
IV. Bombay rats fed on whole carcasses	108								36	5	38.0
Total	592								155		26.2

From these figures it appears that the ship rats are twice as susceptible as the Bombay rats, both series receiving the same quantity

of infected food. It is interesting to note that the same difference in susceptibility between these two classes of rat was found in a series of experiments in which infected fleas were the agents by which plague was transmitted. Of the Punjab rats three times as many were infected as of the Bombay rats, and all died on or before the fifth day. The larger percentage of positive results and the more acute form of the disease are probably largely accounted for by the larger amount of infected material given to the rats. The same consideration may well account for great increase of infections in Series IV compared with Series I.

*Analysis of the post-mortem examinations of the rats which died of plague.*

We have already described in detail the post-mortem appearances which are found in rats which have died from plague in nature. With the exception of two very important points, namely, the distribution of the primary bubo and the condition of the intestinal tract, the pathological lesions seen in our present series were similar to those found in rats naturally infected. Thus subcutaneous congestion was commonly observed, but only in a very few instances (2%) were subcutaneous haemorrhages present. This relative absence of such haemorrhages is a point worthy of note, as in rats naturally infected they were present in 40% examined. The liver showed all the changes which we have already described under the terms "fatty," "mottled," and "granular." The spleen was sometimes enlarged and granular, but its appearance was by no means constant. The kidneys and suprarenal capsules were often found congested. In the thorax the lungs sometimes showed a patchy congestion and even punctiform haemorrhages, but there is no record of a lobar pneumonia having been detected. Pleural effusion of the same clear type as previously described was a very characteristic feature. As regards the microscopical examination of the tissues, plague bacilli were always found in the buboes in very large numbers; in the great majority of cases they were also present in the spleen, while the blood showed organisms in 88% of the cases. The microscopical examination of the tissues, in fact, yielded results exactly the same as those obtained in the naturally infected rats which we have already described.

The above description refers equally well to the 36 animals which died of plague as a result of feeding on the whole carcasses of plague infected rats.

*Distribution of the primary bubo.*

The first great difference between the post-mortem lesions of the rats of these series and those of naturally infected rats is to be found in the distribution of the primary bubo. We have already defined what we mean by a primary bubo and the various appearances which it may present. The same description applies to the bubo found in our present experiments. When, however, we come to analyse the distribution of the bubo, it is seen (*vide* Table II) that in rats infected by feeding by far the commonest situation is the mesentery. In the series fed on soft material, of 109 animals which had buboes, the mesenteric glands were involved in 79 instances (= 72 %), and the cervical glands in 39 (36 %). In the series of rats (Table III) infected by feeding on bony material, namely, the carcasses of plague-infected rats, we find that mesenteric buboes occurred in 76 % of 33 animals which had buboes, and cervical buboes in 31 %. This is in marked contrast to the results obtained in the examination of naturally infected plague rats in Bombay. In a series of 4000 rats (see above, p. 329) no mesenteric buboes were found, nor were any seen in a further series of 1000 which were especially examined with this point in view: on the other hand, 72 % of the animals with buboes had cervical buboes, which occurred only in 32 % of those infected by feeding.

TABLE II.

*Animals fed on soft material (viscera).*

Total number of rats dead of plague	No bubo	Single bubo		Multiple buboes	
119	10 (8·4 %)	98 (82·4 %)		11 (9·2 %)	
		Mesenteric	Submaxillary	Submaxillary and Mesenteric	Axillary and Inguinal
		69 (70·4 %)	29 (29·6 %)	10 (91 %)	1 (9 %)

72·5 % of animals with buboes had a primary mesenteric bubo.

TABLE III.

*Animals fed on whole carcasses (including bones).*

Total number of rats dead of plague	No bubo	Single bubo		Multiple buboes
36	3 (8·3 %)	30 (83·4 %)		3 (8·3 %)
		Mesenteric	Submaxillary	Mesenteric + Submaxillary
		22 (73·3 %)	8 (26·6 %)	3 (100 %)

75·8 % of animals with buboes had a primary mesenteric bubo.

*Condition of intestinal tract.*

We have now to record the second striking difference in the post-mortem lesions between naturally infected rats and those infected by feeding. In the case of naturally infected rats we have already noted (p. 333) that the stomach and intestines show no characteristic change. An analysis of the records of all the rats infected by feeding shows that haemorrhages in the stomach wall were present in 3 % of the cases, and that the intestines were markedly congested in about 27 %. It was also noted that in about a third of the cases (31 %) Peyer's patches were markedly enlarged, showing prominently on the serous surface, and congested and haemorrhagic on the mucous surface, which was very often ulcerated. Smears of these ulcers examined microscopically showed abundant plague-like bacilli.

*Rats found plague-infected on being killed on the 21st day.*

Eleven rats come under this category. They appeared quite well and healthy, but on being killed and examined post-mortem showed undoubted signs of being plague-infected. In five instances there was a submaxillary bubo, in three cases the bubo was in the mesentery, while in one instance a bubo was present in the axilla. The liver was slightly granular in many of the cases. While a microscopical examination of spleen and blood smears failed to reveal the presence of the *B. pestis* in any instance, the bubo almost always yielded a specimen showing a few bacilli. From two of the rats the plague bacillus was isolated by culture or animal test. It would appear, then, that these animals had all suffered from a slight attack of plague, and that they were all tending towards a complete recovery.

In no instance did we come across any pathological condition similar to that which we have described elsewhere as "chronic plague" (vol. vi. p. 530, and below, under Paper XIX, p. 457). It will be seen that with the exception of the two cases in which the organism was isolated, the diagnosis of plague rested on the naked-eye appearances, which in the hands of an expert we have shown elsewhere to be quite reliable.



*Bombay rats fed on urine from human plague cases.*

We have seen above that it is quite possible to infect Bombay rats with plague by feeding them with grossly infected material. We have now to record a series of experiments which were made with the object of testing a theory which is held by not a few plague workers in India, namely, that rats become infected in nature through eating the excreta of plague patients. We fed a large number of rats on the urine of plague cases. The rats used were the wild rats of Bombay, no distinction being made between *M. rattus* and *M. decumanus*.

Each rat received one feeding, from 2 to 3 c.c. of urine being mixed with the food, namely "laya" or parched Indian corn. The urine was collected from 35 cases of acute plague, only four of which recovered (88·5 % mortality). At the same time as the rats were fed a rough estimation of the number of organisms present in the blood of the patients was made, 0·1 c.c. of blood being spread over an agar slope and the resulting colonies counted. Of the 35 cases, 19 had plague bacilli in the blood, the growth obtained on agar varying from a few colonies to a thick layer. The infectivity of the urine was tested by injecting varying quantities subcutaneously into guinea-pigs. It was found that seven of the urines were infective in quantities below 1 c.c., one sample giving plague to a guinea-pig in so small an amount as 0·0001 c.c.

In all 194 rats were fed with this material with the result that not one of them developed plague.

*Summary.*

1. It is possible to infect wild rats of Bombay with plague by feeding them with the viscera of dead plague rats, 21·4 % being susceptible to this method of infection. Bombay rats show a greater immunity to infection by feeding than rats of the same species, which have not been subjected to a plague epizootic.

A series of experiments were also done with *Mus rattus* caught in the Punjab. Of these rats 67·8 % were susceptible. In this series a considerably larger dose of infected material was given.

We have infected a large number (38 %) of wild Bombay rats by feeding them on the whole carcasses of their plague-infected comrades. No difference as regards the post-mortem appearances or the distribution of the primary bubo was found between rats infected in this way and rats infected by feeding on soft viscera.

2. The general pathological lesions found in all rats infected by feeding are, in the main, the same as those found in rats naturally infected. There are, however, two striking differences:—

(a) The distribution of the primary bubo is different. The common site in naturally infected plague rats is in the neck, no mesenteric bubo having been seen out of 5000 post-mortems. In the case of fed rats the common site is the mesentery.

(b) In the case of naturally infected rats the stomach and intestines show no marked pathological change. In the case of fed rats well marked pathological lesions are found in the intestines.

3. It would appear that in nature intestinal infection rarely or never takes place, and that in consequence rats do not become infected by eating the carcases of their comrades.

4. A large series of rats were fed on the urine of plague cases. None of these contracted the disease.

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#### XIV. ON THE SIGNIFICANCE OF THE LOCALITY OF THE PRIMARY BUBO IN ANIMALS INFECTED WITH PLAGUE IN NATURE.

##### CONTENTS.

Introduction : importance of the primary bubo as indicating path of infection.

##### I. Distribution of the primary bubo in plague-infected rats.

- (1) Rats naturally infected.
  - (a) Wild rats in Bombay during the plague season.
  - (b) Wild rats in Bombay during the non-plague season.
  - (c) Wild rats in Arab villages during the plague season.
- (2) Rats infected experimentally by means of fleas.
  - (a) Bombay rats.
  - (b) Ship rats.
  - (c) White rats.
- (3) Rats experimentally infected by feeding on plague material.

##### II. Distribution of the primary bubo in plague-infected guinea-pigs.

- (1) Guinea-pigs naturally infected in plague houses.
- (2) Guinea-pigs infected in the course of the go-down experiments.
- (3) Guinea-pigs infected experimentally by means of fleas.

General summary and conclusions.

##### INTRODUCTION:—IMPORTANCE OF THE PRIMARY BUBO AS AN INDICATION OF THE PATH OF INFECTION.

Almost all observers are agreed in the opinion that the primary bubo develops in the glands in connection with those lymphatics by way of which the plague bacillus has entered the body. Hunter (1904-1906), on the other hand, holds that the primary bubo is merely an enlargement of a group of glands in the course of an acute septicaemic disease and is no indication of the path by which infection has taken place.

There is abundant evidence to show that a primary bubo may develop in the glands which are in lymphatic connection with an area in which the infective material has been inoculated in a breach

of the skin surface while, at the same time, no lesion at the site of inoculation may be observed. Wyssokowitz and Zabolotny (1897) report a number of experiments in which they pricked the hands and feet of monkeys with needles contaminated with plague material. Primary buboes developed in the corresponding glands, but no local reaction was observed. The Austrian Plague Commission (1898) also showed that in the case of wild rats a prick infection in the extremities may give rise to no local reaction while a primary bubo is present in the corresponding group of glands. The Indian Plague Commission (1901) recite several instances in man in which the site of infection was apparent, and in which the bubo developed in the group of lymphatic glands in connection therewith. Further, we ourselves have made a series of experiments, in which fleas taken from septicaemic plague rats were fed on guinea-pigs, the area of skin on which they were allowed to feed being strictly limited and the same for each meal. The following was the method :—

Bombay wild rats were inoculated with a virulent culture of the plague bacillus. Next day the rats were placed separately in flea-proof cages and a number of fleas were put in with them. On the death of the rats the fleas, collected from those rats which showed a marked plague septicaemia, were placed in a glass tube, one end of which was open while the other end was closed with a single layer of fine muslin. About twenty fleas were put into each tube. The hair on a small area of a guinea-pig's skin was removed. The fleas were now allowed to feed on this area through the muslin covering the end of the tube. They were given a morning and an evening meal, the tube being applied for from 10 to 15 minutes on each occasion. In some cases the feeding only took place on a single day, while in other instances the same fleas were fed daily for several days consecutively, the same area of skin, however, being used on each occasion. The details of these experiments, along with some others in which no note was made of the area of skin on which the fleas were fed, will be published in another paper. For our present purpose we have constructed a table which shows in the case of 17 animals the relationship between the area on which the fleas were fed and through which the plague bacilli entered the skin, and the position of the primary bubo. A study of this table (Table I) makes it apparent that in practically every case the primary bubo developed in the group or groups of glands which stood in direct lymphatic connection with this area of skin. The only apparent exception to this generalisation is guinea-pig No. 2,

in which a left cervical bubo formed after the fleas had been fed on the left side of the abdomen just below the costal margin. In this case the bacilli may have passed through the lymphatic filter of the axillary glands without affecting them.

TABLE I.

Animal	Region on which fleas were fed	Position of bubo	Remarks
G.-P. 1	Below the ribs and slightly to right of middle line	Right inguinal and double axillary	Marked local reaction and oedema
G.-P. 2	Left side of abdomen just beneath the costal margin	Left cervical	No local reaction
G.-P. 3	Left side of abdomen above umbilicus	Left axillary	Local reaction present
G.-P. 4	Right side of abdomen just above umbilicus	Right inguinal	Intense local inflammatory reaction
G.-P. 5	Left side of abdomen 1 inch above umbilicus	Left axillary and double inguinal	Local reaction
G.-P. 6	Middle line $\frac{1}{2}$ inch above umbilicus	Double inguinal	Local reaction present
G.-P. 7	Middle line $\frac{1}{2}$ inch above umbilicus	Double inguinal	No reaction
G.-P. 8	Left side just above umbilicus	Left inguinal	Local reaction
G.-P. 9	Right side just above umbilicus	Right inguinal	No local reaction
G.-P. 10	Right side just above umbilicus	Double inguinal	Typical phlyctenule at site of feeding; subcutaneous tissues underneath oedematous and congested
G.-P. 11	Right side just above umbilicus	Right inguinal	Intense local reaction
G.-P. 12	Middle line $\frac{1}{2}$ inch above umbilicus	Right inguinal	No local reaction
G.-P. 13	Middle line of neck	Double cervical	Oedema at site of feeding
G.-P. 14	Middle line of neck	Double cervical	Typical phlyctenule and oedema
G.-P. 15	Middle line, about level of umbilicus	Double inguinal	Typical phlyctenule. Local oedema and thickening
G.-P. 16	Middle line, about level of umbilicus	Right inguinal	Oedema at site of feeding
G.-P. 17	Middle line, about level of umbilicus	Left inguinal and double pelvic	Local pustule and oedema

In passing we may refer to another point of interest in these experiments, namely, the presence in most cases and the complete absence in others of a marked local reaction at the site of feeding. In the case of



guinea-pig No. 4 this local reaction was very intense, the abdominal wall and peritoneum being bound together in one mass of exudate. In the case of guinea-pigs Nos. 10, 14, 15 and 17 typical phlyctenules developed and the subcutaneous tissues underneath were markedly oedematous and congested. While in this connection it is to be remembered that the infection was concentrated on one small area of skin, still we have occasionally observed typical phlyctenules in guinea-pigs which were naturally infected. Three such instances may be given:—

1. A guinea-pig in house No. 3165 Sion Agrivada, in which a dead rat had been found on 16th March, was noticed to be sick on 24th March. Next day three phlyctenules were seen on the right side of the neck and at the same time the cervical glands were felt enlarged. The phlyctenules, situated in the centre of a small hairless patch of skin, were each surrounded by a zone of redness. They exactly resembled the vesicles seen when plague material is rubbed into the skin of a guinea-pig. Six rat fleas were taken off the animal and it was then killed with chloroform. On examination post-mortem the primary bubo was found in the submaxillary region and the organs presented all the typical appearances of plague.

2. A guinea-pig placed in house No. II, 8. 19 Sion Bhandariwada was noticed to be sick. On examination a typical phlyctenule was found on the lower lip and the cervical glands were enlarged. Four fleas were taken on this guinea-pig. On death a post-mortem examination revealed the typical appearances of plague, primary buboes being present in the submaxillary and cervical regions.

3. A guinea-pig placed in house I, 12. 26 Sion Agrivada was found sick, a small phlyctenule was noted on the neck and 26 fleas were taken on the animal. On death an examination showed that the phlyctenule contained pus, and that there were buboes in the neck and other typical signs of plague.

Having then satisfied ourselves that the primary bubo develops in those groups of glands which are in direct lymphatic connection with the area through which the plague bacillus enters the animal organism, we may now proceed to inquire if, by a study of the relative distribution of the primary bubo, on the one hand in animals naturally infected with plague, and on the other hand in animals artificially infected by different means, any evidence can be obtained which will point to any particular method as being the one by which infection takes place in nature.

## SECTION I. DISTRIBUTION OF THE PRIMARY BUBO IN PLAGUE-INFECTED RATS.

(1) *Rats naturally infected*<sup>1</sup>.

(a) *Wild rats in Bombay during the plague season.* This group of rats is made up of 4000 plague-infected rats which were found in the city of Bombay during the plague epizootic of 1906. In Table II no distinction is made between *M. rattus* and *M. decumanus*.

TABLE II.

*Distribution of the primary bubo in wild rats which contracted plague naturally in the city of Bombay during the plague season.*

No bubo	Single bubo			Multiple buboes				Total
610	2956			434				4000
(15.2 %)	(73.9 %)			(10.9 %)				
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	2194	322	440	78	132	180	44	
	(74.3 %)	(10.9 %)	(14.8 %)	(18.0 %)	(30.4 %)	(41.5 %)	(10.1 %)	

N.B. In the animals with multiple buboes the neck glands were affected in 58.5 % of the cases. Out of a total of 3390 rats with buboes, the neck glands were affected in 2448 instances, namely, 72.2 %.

We have elsewhere given a detailed account of the post-mortem examination of these rats, so that for our present purpose we need only give the details referring to the localisation of the primary bubo. The points in this table which are more particularly to be noted are:—

(a) that the neck glands were involved in 74.3 per cent. of the cases with single buboes and in 58.5 per cent. of those with multiple buboes; and

(b) that in the total number of animals which had buboes the neck glands were affected in 72.2 per cent.

<sup>1</sup> There are on record very few observations relating to the distribution of buboes in naturally infected rats which are based on the examination of an adequate number of animals. Buchanan (*Seventh Annual Report of the Local Government Board for Scotland*, 1902, p. 73) notes that inguinal buboes predominate in rats as in men, while Kitasato (*Philippine Journal of Science*, 1. 1906, p. 472) rightly insists that cervical buboes are the most frequent.

(b) *Wild rats in Bombay during the non-plague season.* This group consists of 900 rats, both *M. rattus* and *M. decumanus*, which were found plague infected in the city of Bombay during the months of June, July, August and September, that is to say, at a time when only sporadic cases of plague were occurring amongst rats and men. A reference to Table III,

TABLE III.

*Distribution of the primary bubo in wild rats which contracted plague naturally in the city of Bombay in the off-plague season.*

No bubo 136 (15.1 %)	Single bubo 712 (79.1 %)			Multiple buboes 52 (5.8 %)				Total 900
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	535 (75.1 %)	66 (9.3 %)	111 (15.6 %)	8	13	30	1	

In the animals with multiple buboes the neck glands were affected in 42.4 % of the cases. Out of a total of 764 rats with buboes the neck glands were affected in 557 instances, namely, 72.9 %.

which contains the details relating to this group, will show that the distribution of the primary bubo is practically the same as in the case of the previous group. This correspondence of the position of the bubo in rats during the plague season and in rats during the off-season seems to us to point to the conclusion that the same method of infection obtains during both seasons.

On comparing in tabular form the position of the primary bubo in 100 *M. rattus* and in 100 *M. decumanus*, which had become infected in nature, we find that the distribution is practically the same in both species:—

TABLE III A.

	<i>rattus</i>	<i>decumanus</i>
Submaxillary	54	51
Axillary	7	11
Inguinal	8	5
Pelvic	3	1
Multiple	3	7
No bubo	25	25

(c) *Wild rats in Punjab villages during the plague season.* We have now to consider the distribution of the primary bubo in a group of 288 rats found plague infected in the Punjab villages Dhand and Kasel during the plague epidemic of 1906. These rats were all *M. rattus*.

The details which concern the distribution of the primary bubo are set forth in Table IV. The first point to be noted is the high percentage of cases without bubo in comparison with the wild rats of Bombay. Secondly, we find the same preponderance of neck buboes both in the cases with single and in those with multiple buboes.

TABLE IV.

*Distribution of the primary bubo in wild rats which contracted acute plague naturally in two Punjab villages during the plague season.*

No bubo	Single bubo			Multiple buboes				Total
103	164 (57%)			21 (7·3%)				288
(35·7%)								
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	87	36	41					
	(53·1%)	(21·9%)	(25%)					
				7	9	3	2	

N.B. In the animals with multiple buboes the neck glands were affected in 86% of the cases. Out of a total of 185 rats with buboes the neck glands were affected in 57% of the cases.

## (2) *Rats infected experimentally by means of fleas.*

We have now to pass on to consider the distribution of the primary bubo in three groups of rats which were infected in the laboratory by means of fleas.

In every instance the rats and fleas, which had received their infection from septicaemic plague animals, were confined together in a flea-proof cage. All other sources of infection were rigorously excluded.

TABLE V.

*Distribution of the primary bubo in wild Bombay rats which contracted plague experimentally as a result of being bitten by infected fleas.*

No bubo	Single bubo			Multiple buboes				Total
14	43 (68·3%)			6 (9·5%)				63
(22·2%)								
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	29	12	2					
	(67·5%)	(27·9%)	(4·6%)					
				2	2	2	0	

N.B. In the animals with multiple buboes the neck glands were affected in 66·6% of the cases. Out of a total of 49 animals with buboes the neck glands were affected in 33 instances, namely, 67·3%.

Three different classes of rats were used for these experiments, so that we can divide the observations into three series as follows:—

(a) *Wild rats of Bombay.* This group consists of 63 wild rats of Bombay, no distinction being made between *M. rattus* and *M. decumanus*.

The details concerning the distribution of the primary bubo are given in Table V.

We would draw attention to the following figures in this table:—

(a) the neck glands were involved in 67·5 per cent. of the cases with single buboes and in 66·6 per cent. of those with multiple buboes; (b) out of the total number of animals with buboes the neck glands were affected in 67·3 per cent.

(b) *Ship rats.* This group consists of a small number, namely 25, of rats which had been caught on board ships in Bombay harbour. They all belonged to the species *M. rattus*, and had probably never been subjected to a plague epizootic. They were infected by means of fleas in exactly the same manner as the rats of the preceding experiment. Table VI contains the data which refer to the distribution of the primary bubo in these animals. From this table it is seen that both in those animals with single buboes and in those with multiple buboes the neck glands were the site of the bubo in the majority of cases, and that out of a total of 24 animals which developed buboes the neck glands were affected in 12 instances, namely, 50 per cent.

TABLE VI.

*Distribution of the primary bubo in ship rats which contracted plague experimentally as a result of being bitten by infected fleas.*

No bubo	Single bubo			Multiple buboes				Total
1 (4 %)	21 (84 %)			3 (12 %)				25
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	9	6	6	2	1	0	0	
	(42·8 %)	(28·6 %)	(28·6 %)					

N.B. In the animals with multiple buboes the neck glands were affected in 100 % of the cases. Out of a total of 24 animals with buboes the neck glands were affected in 12 instances, namely, 50 %.

(c) *White rats.* This group is another small group, numbering only 29, consisting of tame white rats, which had been imported from England and had never been exposed to a plague epizootic. They were infected in the laboratory by means of fleas in the same manner as the rats of the two preceding groups.



The data which refer to the distribution of the primary bubo are set forth in Table VII, a reference to which will show that the neck glands were chiefly affected, namely, in 64·3 per cent. of animals with single buboes, 55·5 per cent. of animals with multiple buboes and 61 per cent. of the total number of animals with buboes.

TABLE VII.

*Distribution of the primary bubo in tame white rats which contracted plague experimentally as a result of being bitten by infected fleas.*

No bubo	Single bubo			Multiple buboes				Total
6	14 (48·3 %)			9 (31 %)				29
(20·7 %)	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	9	4	1					
	(64·3 %)	(28·6 %)	(7·1 %)					
				2	3	4	0	

N.B. In the animals with multiple buboes the neck glands were affected in 55·5 % of the cases. Out of a total of 23 animals with buboes the neck glands were affected in 14 instances, namely, 61 %.

(3) *Rats experimentally infected by feeding on plague material.*

In another paper we have considered in detail the results of several series of experiments, in which plague was transmitted to rats by feeding them with plague-infected material. We have seen that as regards the pathological changes, including the distribution of the primary bubo, the result is the same whether the rats are fed on soft material, *e.g.* liver and spleen mixed with the food, or on the whole carcasses of their comrades which have died of plague.

For our present purpose we reproduce a table (Table VIII) in which the data referring to the distribution of the primary bubo are given for 119 rats which became infected with plague as a result of eating the organs of plague-infected animals minced up and mixed with their food.

TABLE VIII.

*Distribution of the primary bubo in wild Bombay rats which contracted plague experimentally as a result of being fed on plague-infected material.*

No bubo	Single bubo		Multiple buboes		Total
10 (8·4 %)	98 (82·8 %)		11 (9·2 %)		119
	Mesenteric	Submaxillary	Submaxillary & Mesenteric	Axillary & Inguinal	
	69	29	10	1	
	(70·4 %)	(29·6 %)	(91 %)	(9 %)	

The first striking fact which emerges from a study of this table is that the inguinal and axillary glands are practically never affected. The solitary instance in which buboes were present in these regions was probably an experimental accident, the animal possibly infecting its feet through cuts made by the sharp edge of the tin in which the food was placed. Secondly, it is remarkable that 70·4 per cent. of the single buboes were situated in the mesentery, the remaining 29·6 per cent. being under the chin. Lastly, it is seen that practically the only situations in which multiple buboes were found were in the submaxillary and mesenteric regions.

*Summary of Section I.*

In three groups of rats naturally infected with acute plague, 72, 73 and 57 per cent. of those with buboes have the primary bubo in the cervical region. In three groups of rats artificially infected with acute plague by the agency of fleas 67, 50 and 61 per cent. have cervical buboes. On the other hand, cervical buboes are found in only 36 per cent. of rats artificially infected by feeding. Mesenteric buboes occur in 72 per cent. of those infected by feeding and are not found in rats infected naturally or by the agency of fleas.

The next table (Table IX) summarises these observations as shortly as possible.

TABLE IX.

*Showing the percentage frequency of buboes in different parts.*

	Naturally infected	Infected by fleas	Infected by feeding
Rats examined	5188	117	119
Rats without buboes	16 %	18 %	8 %
Cervical bubo	72	61	36
Axillary „	23	22	1
Inguinal „	18	35	1
Mesenteric „	0	0	72·5

One may conclude that *the naturally infected rats are not infected by feeding, and that material evidence is here adduced that they are infected by fleas.*

## SECTION II. DISTRIBUTION OF THE PRIMARY BUBO IN PLAGUE-INFECTED GUINEA-PIGS.

We have now to consider the distribution of the primary bubo in three groups of guinea-pigs, namely, (1) a group naturally infected in plague houses; (2) a group infected in the course of the go-down experiments already described (vol. VI. p. 465), in which all the facts pointed to the infection being carried by fleas; (3) a group experimentally infected in the laboratory by means of fleas.

1. *Guinea-pigs naturally infected in plague houses.*

This group is made up of 87 animals, which had been placed in plague-infected houses either running about or in open wire cages standing on the ground. They became infected under natural conditions and the means of infection can only be surmised.

The details of the primary bubo in these animals are given in Table X. In the great majority of instances the neck glands, either submaxillary or cervical, were involved.

TABLE X.

*Distribution of the primary bubo in guinea-pigs which contracted plague in infected houses.*

No bubo 1 (1.1 %)	Single bubo 80 (92 %)			Multiple buboes 6 (6.9 %)				Total 87
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	73 (91.2 %)	7 (8.8 %)	0	5	1	0	0	

N.B. In the animals with multiple buboes the neck glands were affected in every instance. Out of a total of 86 animals which had buboes the neck glands were affected in 79 cases, namely, 91.8 %.

2. *Guinea-pigs which became infected in the go-downs.*

This group is made up of 253 guinea-pigs, which became plague infected in the course of our experiments in specially constructed go-downs. We have already described these observations in detail (vol. VI. p. 450) and have discussed the question of the method by means of which the animals became infected. While it is, therefore, unnecessary to repeat the arguments then brought forward, we may state that all the data

pointed to the conclusion that the rat fleas present in the go-downs were the agents by which the infection was carried from animal to animal.

The details concerning the position of the bubo in these 253 guinea-pigs are set forth in Table XI, from which it is seen that in the great majority of cases the neck glands were the seat of the primary bubo.

TABLE XI.

*Distribution of the primary bubo in guinea-pigs which contracted plague in the go-downs.*

No bubo 3 (1·2 %)	Single bubo 232 (91·7 %)			Multiple buboes 18 (7·1 %)				Total 253
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	212 (91·4 %)	17 (7·3 %)	3 (1·3 %)	13	3	0	2	

N.B. In the animals with multiple buboes the neck glands were affected in every instance. Out of a total of 250 animals which had buboes the neck glands were affected in 230 cases, namely, 92 %.

### 3. Guinea-pigs which were experimentally infected in the laboratory by means of fleas.

This group is made up of 108 guinea-pigs which became plague infected in the course of experiments of the same nature as those with rats already referred to.

The details concerning the distribution of the primary bubo in these animals are given in Table XII, from which it is once more seen that the neck glands were affected in the large majority of cases.

TABLE XII.

*Distribution of the primary bubo in guinea-pigs which contracted plague experimentally as a result of being bitten by infected fleas.*

No bubo 1 (0·9 %)	Single bubo 89 (82·4 %)			Multiple buboes 18 (16·7 %)				Total 108
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	79 (88·8 %)	10 (11·2 %)	0	12	4	0	2	

N.B. In the animals with multiple buboes the neck glands were involved in every instance. Out of a total of 107 animals which had buboes the neck glands were affected in 97 cases, namely, 90·6 %.

*Summary of Section II.*

The following table (Table XIII) summarises very shortly the distribution of the buboes in these three groups of guinea-pigs.

TABLE XIII.

*Showing the percentage frequency of buboes in different parts.*

	Naturally infected	Infected in go-downs	Infected by fleas
Guinea-pigs examined	87	253	108
Without buboes	1 %	1 %	1 %
Cervical bubo	92	92	91
Axillary „	1	3	6
Inguinal „	14	13	22
Mesenteric „	0	0	0

The close correspondence between the distributions of the buboes in the three groups of animals lends support to the conclusion that the mode of infection was the same in all cases. It would follow that, both in the experimental go-downs and in the plague-infected houses, the infection was conveyed to the guinea-pigs by fleas.

*General Summary.*

Cervical buboes preponderate, on the one hand, in naturally infected rats and in guinea-pigs infected by being placed in plague-infected houses and also in rats and guinea-pigs artificially infected with fleas. In rats artificially infected by feeding mesenteric buboes are the most frequent, whereas in upwards of 5000 naturally infected rats in not a single case was a mesenteric bubo present. It may, therefore, be concluded that rats in nature are not infected by feeding on plague-infected material, but probably by the agency of fleas.

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XV. FURTHER OBSERVATIONS ON THE TRANSMISSION OF PLAGUE BY FLEAS, WITH SPECIAL REFERENCE TO THE FATE OF THE PLAGUE BACILLUS IN THE BODY OF THE RAT FLEA (*P. CHEOPIS*).

We have previously (vol. vi. p. 446) shown that plague may be transferred from rat to rat by the transference of fleas from a septicaemic to a healthy animal. We propose in the present communication to deal with the following questions:—

- I. The fate of the plague bacillus in the body of the rat flea.
- II. Whether a single rat flea can transmit the infection.
- III. Whether both male and female rat fleas can transmit the infection.
- IV. Whether other species of fleas can transmit the infection.
- V. The mechanism by which the flea infects a healthy animal.

I. FATE OF THE PLAGUE BACILLI IN THE BODY OF THE RAT FLEA (*P. cheopis*).

1. *Capacity of Flea's Stomach.*

We have already (vol. vi. p. 486) described the physiological anatomy of the mouth parts and of the alimentary canal of the Indian rat flea, *Pulex cheopis*. We have described how the blood is sucked up from the wound made by the pricking apparatus of the insect through the mouth into the pharynx, how it is then passed down the pharynx and oesophagus by a successive wave of contraction of the muscles from before backwards, and how from the oesophagus it passes into the stomach, at the anterior end of which is a valvular arrangement called "the gizzard." The stomach is a pear-shaped organ occupying a considerable part of the abdomen of the insect. That it is capable of containing a considerable amount of blood is apparent from the

observation that after a flea has had a meal it is seen that nearly the whole of the abdomen is filled with a bright red mass.

We have made an endeavour to measure as accurately as possible the average capacity of the stomach when filled with blood. Two methods were employed, namely, (a) direct measurement, (b) indirect estimation.

(a) *Direct measurement of Flea's Stomach.*

Healthy fleas, taken from Bombay rats, were starved for 12 hours, and at the end of that time fed on healthy animals. The stomach was then dissected out whole and floated in normal salt solution, adherent tracheae and the fat body being removed. Under these conditions the organ assumes a regular ellipsoidal form and direct measurement can be employed. The microscope was adjusted to a magnification of exactly 100 diameters and the image of the stomach floating in salt solution was projected on paper. The outline was traced, and then measured, the measurements being put through the "Simpson's first rule." The method was controlled by taking globules of mercury of known volume, and putting them through the same process. It was found to be accurate to 1/10 of a cubic millimetre but not to one hundredth. Working in this way we have obtained the following results:—

(a) Volume of very full and distended stomach from large flea, 0.7—0.75 c.mm.

(b) Volume of full stomach of average sized flea, 0.38—0.48 c.mm.

It is to be noted that this method gives the total volume of the stomach, including the stomach walls. It is an outside estimate.

(b) *Indirect estimation of capacity of Flea's Stomach.*

In this method a comparison was made between the tint of a solution of the contents of several stomachs and that of standard solutions of carbonic oxide haemoglobin.

A series of 10 standards, namely 1 to 10 p.c., of dilutions of rats' blood, through which carbonic oxide had been passed, was prepared. These were put up in calibrated capillary tubes, and arranged side by side on a card. Five fleas, which had just fed, were then dissected and their stomach contents dissolved in a known quantity of distilled water, and CO passed through. The resulting fluid was introduced into a capillary tube of the same calibre as the standards, and a comparison made with the standards. Having ascertained the standard tube to which it corresponded in tint, an easy calculation determined the volume of blood in the stomachs.

The following results were obtained :—

- (a) Large distended stomach — average of 5 stomachs = 0·3 c.mm.
- (b) Large distended stomach — average of 5 stomachs = 0·25 c.mm.
- (c) Small half filled stomach — average of 5 stomachs = 0·12 c.mm.

It will be noted that these estimations are considerably lower than those obtained by direct mensuration. This result was anticipated, as in the direct method the walls of the stomach are included, and the result is therefore certainly too high. The haemoglobin method, on the other hand, gives too low an estimate, as it is based on a comparison with whole fresh blood in the standards, while the stomach of the flea contains, mixed with the fresh blood of the last meal, a large proportion of residuum of darkly coloured semi-digested material of the previous meals. It is also impossible to arrest the process of digestion immediately after feeding. For our present purpose, therefore, we shall not be greatly in error if we consider the average volume of blood capable of being accommodated in the stomach of a rat flea at each meal to be about 0·5 c.mm.

## 2. *Number of Plague Bacilli taken into the Stomach.*

In our first report (vol. VI. p. 519) we have shown that the blood of a plague-infected rat may contain an enormous number of plague bacilli, even as many as 100,000,000 per c.c., having been found before death. If, therefore, a rat flea imbibed the blood of such a rat, it would receive into its stomach, allowing the capacity of this organ to be 0·5 c.mm., 5000 plague germs. It is further evident that a flea which imbibed the blood of a rat containing 10,000 or more plague germs per c.c. would take some bacilli into its stomach. From the table published in vol. VI. p. 521 it will be seen that about two-thirds of plague rats, either before death or immediately after death, contain more than this number of germs in their blood, so that fleas would have ample opportunity of taking bacilli into their stomachs.

### *Summary.*

1. *The average capacity of a rat flea's stomach has been approximately estimated to be 0·5 c.mm.*
2. *A rat flea imbibing blood from a plague-infected rat might receive as many as 5000 germs into its stomach.*
3. *Fleas feeding on a large proportion of plague-infected rats just before death would imbibe some plague bacilli.*

### 3. *Multiplication of Plague Bacilli in the Stomach of the Rat Flea*<sup>1</sup>.

Several observers have previously noted what they consider to be a multiplication of plague bacilli in the stomachs of fleas which had fed on plague-infected rats. The evidence of multiplication having taken place was in all instances the observation that the contents of the flea's stomach, on being squeezed out on a slide and stained, contained abundant bipolar stained bacilli which stained well and showed no involution forms. No account was taken of the interval between the ingestion of the septicaemic blood and the examination of the stomach contents. It is obvious that such evidence of multiplication is very incomplete, inasmuch as we know that soon after a flea has filled its stomach, absorption begins and the blood in the stomach is greatly reduced in volume, finally becoming a thick tarry mass.

The number of bacilli seen in a preparation of the stomach contents at this time will appear to be greatly in excess of the number which is to be found in any preparation of rats' blood, so that a false impression that multiplication has taken place might be conveyed.

As time goes on, the tarry mass in the stomach passes on into the rectum, this process being greatly accelerated by the fresh blood which the flea takes into its alimentary canal. We have already drawn attention to the habit which the flea, in common with other blood-sucking insects, possesses, of squirting bright red blood from the anus during the operation of sucking. It is evident that the whole of the alimentary canal is being subjected to a regular process of washing out. If then fleas, taken from septicaemic plague rats, are fed on healthy animals, a fresh animal being used each day, so that there is no chance of the fleas taking a second supply of bacilli into their stomachs, and if

<sup>1</sup> When we talk of "plague bacilli" in the stomach of a flea, we mean that there was present an organism which was microscopically identical with the plague bacillus. The evidence that this organism is really the plague bacillus is based mainly on the observation that no such organism has ever been found by us in the stomach contents of fleas taken from healthy rats. It is only when fleas have fed on plague-infected rats containing plague bacilli in their blood that a bipolar stained bacillus is found in their stomach contents. We have also the evidence that the faeces of fleas taken from plague-infected rats gives plague to animals and that the fleas themselves can transmit the disease to other animals.

We have already described (vol. vi. p. 492) the method by means of which the abdominal organs of a flea are dissected out. After these organs have been separated from one another the stomach is opened with a sharp needle and its contents, mixed with a little salt solution, smeared over the slide.



at the end of several days the stomach contents are found to be crowded with plague bacilli, we can only conclude that multiplication of the original bacilli has taken place. Had multiplication not taken place the successive diluting and sweeping out processes which had been going on would certainly have cleared out the stomach of most of the bacilli originally taken in.

We have accordingly made a large series of observations, which had as their object the determination of the presence or absence of abundant plague bacilli in the stomach contents of fleas at varying intervals after they had imbibed septicaemic blood, the fleas being fed in the meantime on the blood of healthy animals.

These observations were made during the months of December to April, which is the epizootic and epidemic plague season in Bombay. The method was as follows. Bombay rats were inoculated with virulent cultures of plague bacilli. When sick they were placed separately in flea-proof cages and a number of fleas, which had been taken from wild Bombay rats, were put in with them. The rats were removed from the cages as soon after death as possible, any fleas which were still on them being caught and examined. For our further observations only those cages were used which had contained rats showing a well-marked septicaemia at death. The same day a fresh healthy rat or guinea-pig was put into the cage and kept in for 24 hours. It was then removed and segregated, some of the fleas which were caught on it being dissected, while the others were returned to the cage. The same process was repeated each day. Thus, on each succeeding day, the fleas were supplied with the blood of a healthy animal so that there could be no question of a fresh infection with plague bacilli. The fleas which were removed from the cage each day were dissected: a smear of the stomach contents was made, stained with thionin-blue and examined microscopically, the presence or absence of abundant plague bacilli being noted.

The results of these examinations have been brought together on the basis of the interval between the probable time at which the fleas imbibed the plague bacilli and the time at which the examination of the stomach contents was made. It is, of course, impossible to determine exactly when the fleas took their last meal containing bacilli, as they were in the same cage with the sick rats for from 12 to 48 hours before the death of the latter. On several occasions we made attempts to fix definitely the hour at which the fleas took the bacilli into their bodies. This was done by taking rats which were evidently very ill with plague,



confining their movements and allowing fleas to feed on them through a layer of muslin covering the end of a glass tube. It was, however, soon found that a rat sick with plague will not stand handling as it dies almost immediately from heart failure. This attempt, therefore, had to be abandoned, as it was only on the rarest occasions that the fleas were able to obtain blood containing any plague bacilli.

While, then, in these observations we cannot definitely fix the time at which the fleas took the plague bacilli into their stomachs, it is more than probable that the great majority of these, in the stomachs of which bacilli were found in large numbers, had fed on septicaemic blood within twelve hours of the rat's death. For we know that fleas feed frequently when left to their own inclinations, and that they are especially fond of animals which are sick and unable to clean themselves.

TABLE I.

*Results of examination of rat fleas as to presence of abundant plague bacilli in stomach contents, day by day after their being taken from septicaemic rats, the fleas in the meantime being fed on healthy animals. December to April.*

Interval between ingestion of septicaemic blood and examination of flea	Number of fleas dissected	Number of fleas found with abundant plague bacilli in stomach contents	Percentage of fleas with abundant plague bacilli in stomach contents
1 day	54	22	47
2 days	16	6	38
3 "	24	9	37
4 "	28	15	53
5 "	37	8	21
6 "	33	5	15
7 "	25	3	12
8 "	18	3	16
9 "	13	0	0
10 "	15	1	7
11 "	17	2	12
12 "	22	2	9
13 "	16	0	0
14 "	13	0	0
15 "	6	0	0
16 "	16	0	0
17 "	14	0	0
18 "	—	—	—
19 "	14	0	0
20 "	15	1	7
21 "	19	0	0
22 "	—	—	—
23 "	13	0	0

For our present purpose, therefore, we have taken 12 hours before the death of the rat as the time when the fleas imbibed the bacilli, and as most of the rats died during the night the day on which they were found dead is reckoned as one day after the fleas took the germs into their stomachs, the next day as two days, and so on. Throughout this paper we shall talk of "first day fleas," "second day fleas," etc., and the meaning which is to be given to these expressions is as we have explained.

Table I contains the results of the present observations. From this table it is seen that day by day, up to the 12th day, a considerable percentage of the fleas examined contained abundant plague bacilli in the contents of their stomachs, and that, while all fleas examined on the 13th to 19th days contained no bacilli, a single specimen out of 15 dissected on the 20th day showed abundant germs in its stomach.

*Summary.*

*Fleas were fed on plague-infected rats until the death of these animals. They were afterwards fed on healthy animals. A number were dissected each day for 23 days. In a certain proportion abundant plague bacilli were found in the stomach contents up to the 12th day and in one instance on the 20th day. We have good evidence in this observation that multiplication of plague bacilli may take place in the flea's stomach.*

4. *Estimation of approximate proportion of Fleas in the Stomach of which multiplication of Plague Bacilli takes place.*

We have now to pass on to consider if any estimation can be made, even approximately, of the proportion of fleas in the stomachs of which multiplication of the plague bacilli takes place. In this connection it is necessary to point out that, of the fleas which are caught on a rat dead of plague, it is always impossible to say how many have imbibed blood containing bacilli, and how many, not having fed at all, or having fed at a time when no plague bacilli were present in the blood, did not take bacilli into their stomachs. If, then, a certain number of fleas, which have been taken from a septicaemic plague rat, be examined day by day and the number containing abundant plague bacilli in their stomachs be noted we can calculate the percentage of those in which multiplication of the bacilli has taken place only in terms of the total number which was exposed to infection and not in terms of those which

took bacilli into their stomachs. Further, it is obvious that the number of fleas found infected, that is to say, containing abundant plague bacilli in their stomachs, may be influenced by the interval which has elapsed between the time of their feeding on septicaemic blood and the time of examination. And again it may be influenced by the season of the year at which the experiments were carried out.

The observations which were conducted with the object of answering these questions were made in exactly the same manner as those we have just described, that is to say, the stomach contents of fleas, which had been in contact with septicaemic plague rats, were examined day by day after the death of the rat, the fleas in the meantime being fed on healthy animals. In all three series of experiments were made, each at a different season of the year.

(a) The first series was carried out between the months of December and April, the plague season in Bombay. Table I contains the results, and to those we would now refer.

It is seen from this table that for the first four days after the fleas have had an opportunity of feeding on septicaemic blood 43 per cent. of them contain abundant plague bacilli in their stomachs; that from the 5th to the 12th day 13 per cent. were still infected; and that, while the stomach contents of all those examined between the 13th and 19th day were free from germs, still on the 20th day abundant bacilli were found in the stomach contents of one out of 15 fleas dissected.

TABLE II.

*Results of examination of fleas as to the presence of abundant plague bacilli in stomach contents, day by day after their being taken from septicaemic rats, the fleas in the meantime being fed on healthy animals. May 7th to June 9th.*

Interval between ingestion of septicaemic blood and examination of flea	Number of fleas dissected	Number of fleas found with abundant plague bacilli in stomach contents	Percentage of fleas with abundant plague bacilli in stomach contents
2 days	27	0	0
3 "	36	5	15
4 "	36	0	0
5 "	24	2	8
6 "	12	0	0
7 "	21	3	14
8 "	20	0	0

(b) The second series was made between May 7th and June 9th, 1906, when epidemic plague had practically died down in Bombay.

The results of this series are given in Table II, from which it is seen that while some fleas examined contained abundant plague bacilli in their stomachs, this percentage was very much less than in the first series; that only on three out of eight days were infected fleas found; and that none were got after the 7th day.

(c) The third series of observations was made between 17th June and 3rd Sept., 1906, a season when epidemic plague is low, but in which there has been observed in various epidemics in Bombay a slight recrudescence, especially in August.

The results of this series are given in Table III, from which it is seen that only on the 2nd, 3rd, and 5th days were infected fleas found, and that the percentage of infected ones on the total examined was small in comparison with the figures obtained during the plague season.

TABLE III.

*Results of examination of fleas as to the presence of abundant plague bacilli in stomach contents, day by day after their being taken from septicaemic rats, the fleas in the meantime being fed on healthy animals. June 17th to September 3rd.*

Interval between ingestion of septicaemic blood and examination of flea	Number of fleas dissected	Number of fleas found with abundant plague bacilli in stomach contents	Percentage of fleas with abundant plague bacilli in stomach contents
2 days	12	2	16
3 „	78	12	16
4 „	41	0	0
5 „	23	1	4
6 „	7	0	0

In the next table (Table IV) are brought together the results of the examination of all fleas dissected between the 2nd and 6th day inclusive. This table shows that during the epidemic plague season the number of fleas found infected between the days mentioned is six times the number found in the non-epidemic season. It is also noteworthy that during

TABLE IV.

*Comparison of number of fleas found infected between the 2nd and 6th days after ingestion of septicaemic blood at three seasons of the year.*

Season of year	Number of fleas examined	Number of fleas found with abundant plague bacilli in stomach contents	Percentage of fleas with abundant plague bacilli in stomach contents
December to April	138	43	31·2
May 7th to June 9th	135	7	5·2
June 17th to September 3rd	161	15	9·3

the season when plague is liable to show a slight recrudescence the number of infected fleas was nearly double the number found in May and first half of June.

*Summary.*

1. *The percentage of fleas, which have been taken from septicaemic plague rats, found with abundant plague bacilli in their stomach contents varies with the season of the year, being six times greater in the epidemic season than in the non-epidemic season.*

2. *In the epidemic season this percentage was greatest for the first 4 days, but a certain number was found infected up to the 12th day. On one occasion the stomach contents of a 20th day flea were found full of plague bacilli.*

3. *In the non-epidemic season no flea was found with plague bacilli in its stomach after the 7th day.*

5. *Presence of Plague Bacilli in the Rectum and the Faeces of Infected Fleas.*

In the paper dealing with the physiological anatomy of the flea we have seen that the blood after the end of the digestive process in the stomach passes into the rectum as a thick, slimy, dark-red mass and appears at the anus as minute, dark-red or black tarry droplets. We have examined the contents of the rectum for the presence of plague bacilli in the course of the systematic daily dissections of fleas which were made for the observations which have been already described. On very many occasions we have seen the rectal contents crowded with plague bacilli.

As regards the faeces we have examined them both microscopically and by animal tests for the presence of plague bacilli. The following are the results:—

(a) *Microscopically.*

A number of infected fleas are put into a test-tube; the mouth of the tube is covered over with a glass slide and the tube turned upside down. The fleas are then seen to run about freely over the surface of the slide, and if left for a short time they deposit a considerable amount of faecal matter on this surface. On fixing and staining this smear of faeces it is found that the whole preparation is covered with plague bacilli, their arrangement suggesting that they had been smeared about by the legs of the fleas as they move from place to place.



(b) *Subcutaneous inoculation into Guinea-pigs.*

This series of experiments was made by injecting subcutaneously into guinea-pigs an emulsion of the faeces of fleas taken from plague-infected rats. A number of fleas, between 20 and 30, which had been taken from rats dead of septicaemic plague, were put into a glass tube, one end of which was covered in with a layer of fine muslin. Through this layer of muslin the fleas were allowed to feed on a healthy guinea-pig. After they had fed for from 10 to 15 minutes the muslin was always found soiled with faeces. The faeces were then emulsified, and the emulsion injected subcutaneously into guinea-pigs. We have injected 15 guinea-pigs with the faeces of infected fleas collected in this manner. Out of these animals 10 contracted plague. Of these 10 guinea-pigs five died of typical plague between the 3rd and 11th days. The other five, being judged to be plague infected, were killed with chloroform between the 8th and 13th days. In these cases the lesions consisted of swelling and oedema at the site of inoculation; a bubo in the corresponding set of glands; and in two instances coarse granules in the spleen and liver. A culture of the plague bacillus was got from each of these animals.

It is to be noted that these experiments were made during the month of October, 1906, when plague is only sporadic both in rats and man.

(c) *Cutaneous inoculation into Guinea-pigs.*

We have made a further series of experiments to test the infectivity of the faeces of infected fleas. The faeces were collected on muslin in the same manner as we have described for the preceding experiments. An emulsion made with sterile broth was rubbed into a scarified surface on the abdomen of a guinea-pig. Of nine animals inoculated with this material two died of typical plague.

*Summary.*

*The rectal contents and faeces of fleas taken from septicaemic plague rats often contain abundant virulent plague bacilli.*

6. *Absence of Plague Bacilli from other Parts of the Body of infected Fleas*<sup>1</sup>.

We have already seen that it can easily be demonstrated that abundant plague bacilli are present in the stomach and rectum of plague-infected fleas and that multiplication of these bacteria takes place in the stomach. On rare occasions a few bacilli have been seen in the oesophagus, but only in those cases in which the flea has been killed immediately after feeding on septicaemic blood.

As regards other regions of the body we may say at once, that out of more than a hundred infected fleas which have been examined specially for this purpose on no occasion have any plague bacilli been found outside of the organs already mentioned. No infection of the body cavity has been seen, and although particular attention was paid to the salivary glands, nothing at all resembling a plague bacillus has ever been detected in them.

These dissections were carried out on fleas which had come off septicaemic rats at intervals varying from a few hours to several days, the fleas in the meantime being fed on healthy animals.

7. *Duration of Infectivity of Fleas after they have imbibed septicaemic Plague Blood.*

We have already seen that plague bacilli multiply in the stomachs of fleas, and that they can be found there in abundant numbers for at least twelve, and even for twenty days after the insect has imbibed septicaemic blood. It, therefore, was of some importance to test how long fleas taken from plague-infected rats remained infective, that is to say, were capable of transmitting the infection to healthy animals. With this object in view three series of experiments were done.

*Series 1.* Fleas were infected by placing them in company with Bombay rats, which had received subcutaneous injections of a virulent culture of plague, and in which at death a considerable number of plague bacilli were found in the blood. The fleas were removed from the cage as soon after the death of the rat as possible. They

<sup>1</sup> In the summary of previous observations on the relation of the plague bacillus to the flea already published (vol. vi. p. 425) we omitted to note that Buchanan (Seventh Annual Report of the Local Government Board for Scotland, 1902, p. 73) found "enormous numbers" of plague bacilli in the "stomach and other parts of the body" of a flea taken from a rat dead of plague in Glasgow.

were then transferred to a clean flea-proof cage<sup>1</sup> with a small wire cage in the centre, in which a healthy white rat or guinea-pig was placed. The animal was left in the cage for 24 hours. It was then removed, cleaned of fleas, and segregated. The fleas were counted, and returned to the cage, into which a fresh animal was put. This proceeding was repeated each day until no more fleas could be taken on the animals.

Working in this way we have made 19 experiments, the duration of each observation varying from 6 to 15 days. In all 195 animals were exposed to infection and of these animals 29 guinea-pigs developed plague and died.

Table V gives the details of these observations. From this table it is seen that an average of 97 fleas taken from septicaemic rats were added originally to each cage; that the number of these fleas which could be recovered from the guinea-pigs gradually diminished day by day, until after 15 days an average of only 0·5 per cage was taken.

TABLE V.

*Experiments to ascertain the duration of infectivity of fleas taken from plague rats.*

SERIES 1. 10th March to 26th April.

Interval between ingestion of septicaemic blood by fleas and exposure of animals to infection	Average number of fleas caught on animals; average original number put in being 97	Number of animals exposed to infection	Number of animals which died of plague
1 day	32	10	6
2 days	28	10	4
1 & 2 "	—	9	5
3 "	26	17	3
4 "	18	17	2
3 & 4 "	—	2	0
5 "	12	19	2
6 "	8	19	1
7 "	5	18	3
8 "	5	18	2
9 "	6	14	0
10 "	4	14	1
11 "	3	9	0
12 "	2	8	0
13 "	2	5	0
14 "	1	4	0
15 "	0·5	2	0

<sup>1</sup> The reader is referred to the picture facing page 435 in vol. vi. The present cage was of the same pattern but was a little smaller and had only one wire cage inside.

Further, it is seen that of the animals exposed to the bites of first and second day fleas, or to fleas of both these days, a little more than half died of plague; that on and after the 3rd day the proportion of animals which became infected fell considerably, but that on each day, except the 9th, up to the 10th some of the animals contracted the disease. Lastly, it is seen that no animal after the 10th day became infected.

Tested in this way we can say that the fleas remained infective for at least ten days, and the probability of their being infective diminished *pari passu* with the number which could be caught on the animal, and with the length of the interval from the time of feeding the fleas on the infected rat.

It is to be noted that this series of observations was made during the height of the plague epidemic season, 10th March to 26th April.

*Series 2.* In this series a technique similar to that used in the preceding experiments was employed, the only difference being that, instead of a number of small cages, a single long cage was substituted.

This cage, of the same pattern as that already described, contained inside eight wire cages, which held the experimental animals. It had been used principally as a breeding place for rat fleas, having been started for this purpose on 16th December, 1905, with a stock of 40 fleas. On the 20th April, 1906, when the present experiment was begun, there were several hundred fleas in the cage, all bred from the original forty. On this date six Bombay rats, which had been inoculated on the previous day with a virulent culture of *B. pestis*, were placed separately in the small wire cages. Two of these rats were found dead on the 21st April and the remaining four on the 22nd. The blood of all these rats contained abundant plague bacilli.

On the 22nd April a healthy guinea-pig was put into one of the wire cages and left in for 24 hours. It was then removed, thoroughly cleared of fleas, and segregated. The majority of the fleas were returned to the cage, but a few were dissected and the stomach contents examined microscopically as to the presence or absence of abundant plague bacilli. The same procedure was carried out day by day for 25 days, the only variation being, that from the 10th to the 18th day inclusive, two fresh guinea-pigs instead of one were daily exposed to infection in the cage. From Table VI, which contains the results of these observations, it is seen that up to and including the 8th day a considerable proportion of the fleas examined were found infected; that from the 9th to the 12th day although infected

fleas were still found, their number had diminished, and that from the 13th day onwards only on one occasion, namely, the 20th day, were plague bacilli found in the stomach contents of any of the fleas examined. As regards the fate of the guinea-pigs, it is seen that with one exception, namely, the animal exposed on the 6th day, all guinea-pigs exposed to infection up to and including the 11th day

TABLE VI.

*Experiments to ascertain the duration of infectivity of fleas fed on plague-infected rats.*

SERIES 2. 20th April to 12th May.

Mean daily temperature 83° F.				
Interval between ingestion of septicaemic blood and exposure of animals to infection	Number of fleas examined	Number of fleas with plague bacilli in stomach contents	Number of guinea-pigs exposed to infection	Number of guinea-pigs which died of plague
1 day	—	—	1	1
2 days	10	3	1	1
3 "	15	5	1	1
4 "	15	8	1	1
5 "	15	2	1	1
6 "	13	8	1	0
7 "	14	2	1	1
8 "	18	3	1	1
9 "	13	0	1	1
10 "	15	1	2	2
11 "	17	2	2	2
12 "	22	2	2	0
13 "	16	0	2	0
14 "	13	0	2	0
15 "	6	0	2	1
16 "	16	0	2	0
17 "	14	0	2	0
18 "	14	0	2	0
19 "	—	—	1	0
20 "	15	1	1	0
21 "	19	0	1	0

died of plague; and that after this day, only one animal, namely, the one put in on the 15th day, contracted the disease. When we compare day by day the number of fleas which contained bacilli in their stomachs and the number of animals which became infected we find that both diminished equally. The total number of fleas in the cage did not apparently decrease, being kept up by the breeding which was going on. It would appear, therefore, either that the fleas which had originally imbibed bacilli died out as in the previous experiment,



or that the bacilli disappeared from their stomachs. It is to be noted that this observation was made at the end of April and beginning of May, 1906, when the daily mean temperature was about 83° F. and when plague, although on the decline, was still epidemic in the city.

*Series 3.* This experiment was carried out in a similar manner to the last, but was made about five weeks later when the daily mean temperature was about 88° F., and when the epidemic in Bombay had practically ceased.

The same cage was used as was employed for the last observation. On the 26th May, fleas being very abundant in the cage, eight rats which had been inoculated on the day previous with a virulent culture of *Bacillus pestis* were put separately into the wire cages. On the 27th five rats were found dead: in the blood of four of these rats there were abundant plague bacilli, but the blood of the fifth was free from organisms. On the 28th May two of the remaining rats were dead, both with abundant plague bacilli in the blood. The last rat was taken out.

The same procedure was followed as in the previous experiment,

TABLE VII.

*Experiments to ascertain the duration of infectivity of fleas fed on plague-infected rats.*

SERIES 3. 20th May to 11th June.

Mean daily temperature 88° F.

Interval between ingestion of septicaemic blood and exposure of animals to infection	Number of guinea-pigs exposed to infection	Number of guinea-pigs which died of plague
1 day	2	2
2 days	2	0
3 "	2	1
4 "	2	0
5 "	2	1
6 "	2	0
7 "	2	1
8 "	2	0
9 "	2	0
10 "	2	0
11 "	2	0
12 "	2	0
13 "	2	0
14 "	2	0
15 "	2	0

with the exception that two guinea-pigs were put into the cage each day and all the fleas were returned to the cage, none being kept for dissection.

The result of this experiment is detailed in Table VII, from which it is seen that only on four days, namely, the 1st, 3rd, 5th and 7th day, did either of the animals contract plague, and that only on one occasion, namely, the 1st day, did both the animals become infected.

*Summary.*

1. One series of experiments made during the epidemic plague season to test the duration of infectivity of rat fleas fed on septicaemic rats' blood showed that these fleas could remain infective for at least ten days. This series was made in separate cages with a limited supply of fleas.

2. A second series also made during the epidemic season, but in a single large cage in the presence of a large number of fleas, gave the time that fleas might remain infective as 15 days.

3. In a third series of experiments conducted under the same conditions as the second series but during the non-epidemic season, the fleas remained infective for only seven days, and, further, far fewer (one-third instead of two-thirds) animals than in the second series contracted the disease.

II. EXPERIMENTS TO SHOW THAT A SINGLE RAT FLEA (*P. cheopis*), TAKEN FROM A PLAGUE-INFECTED RAT IS RARELY ABLE TO CAUSE INFECTION OF A HEALTHY ANIMAL.

In all the experiments on flea transmission which we have carried out, a considerable number of fleas has been used on each occasion.

It was therefore of some interest to ascertain if a single flea taken from a septicaemic plague rat could transmit the infection.

Infected fleas were obtained in the manner we have already described. They were then transferred to healthy white English rats in clean flea-proof cages, one flea being added to each cage.

Sixty-seven experiments were made in this manner, but in only one instance was there a successful transference of the disease. From the result of our dissections of fleas taken from plague-infected rats (*vide supra*) we can estimate that about half of these 67 fleas would contain plague bacilli in their stomachs. It would appear, therefore,

that the chances of a non-immune animal, such as the tame white rat, contracting plague after being bitten by a single infected flea are remote.

### III. EXPERIMENTS TO SHOW THAT BOTH MALE AND FEMALE RAT FLEAS (*P. cheopis*) CAN TRANSMIT PLAGUE.

A series of experiments was made to ascertain if both the male and female of *P. cheopis* could transmit plague from animal to animal. Fleas taken from septicaemic plague rats were chloroformed, examined under the low power of the microscope, and separated into male and female. Each lot, of from 15 to 25 individuals, was added to a fresh guinea-pig in a flea-proof cage. Working in this manner we have made six experiments with males and six with females, with the result that one animal in each series became plague infected. The experiment was stopped after this success had been achieved.

### IV. EXPERIMENTS TO ASCERTAIN IF FLEAS OF SPECIES OTHER THAN *P. cheopis* CAN TRANSMIT PLAGUE FROM ANIMAL TO ANIMAL.

We have not been able to procure in Bombay any other species of rat flea except *P. cheopis* in numbers sufficient for experimental purposes. Our experiments in this direction have therefore been confined to two species not commonly found on rats, namely *P. felis*, the common dog and cat flea, and *P. irritans*, the human flea, and to two experiments with *Ceratophyllus fasciatus*, the common rat flea of Europe, which is found in small numbers on rats in the Punjab.

#### (a) *Experiments with Cat Fleas (P. felis).*

These fleas were for the most part collected from guinea-pigs allowed to run about in places where goats were tethered. The fleas were then placed on Bombay rats which had been inoculated on the previous day with a virulent culture of *B. pestis*. On the death of the rat, if a good septicaemia was present, each flea taken from the cage was placed in a separate test tube. It was then examined by means of a hand lens so as to exclude any rat flea which might be present. The method used for this differentiation is described elsewhere (p. 446). About 20 fleas were then added to a fresh guinea-pig in a clean, flea-proof cage. Working in this way we have carried out 27 experiments with cat fleas without any suc-

cessful transmission taking place. It may be added that these experiments were made during the height of the plague epidemic in Bombay.

(b) *Experiments with Human Fleas (P. irritans).*

The supply of human fleas was got from houses which had been recently evacuated by the inhabitants. A man with bare legs is sent into the house, and remains in for a few minutes. On his coming out his legs are seen to be covered with fleas, which are easily picked off and put into test tubes. The great majority of these fleas belong to the species *P. irritans*, but a few rat fleas can also be taken in this way. The fleas are then infected in the same manner as in the previous experiments. After being infected each flea is examined with a lens to make certain that no rat fleas are introduced. The infected fleas, about 20 for each experiment, are put into a flea-proof cage along with a healthy fresh guinea-pig.

Working in this manner, during the months of April and May, 1906, we have completed 38 experiments with *P. irritans*, with the result that three of the guinea-pigs became plague infected. It is evident, therefore, that while this flea is able to transmit the infection it does not transmit it as readily as *Pulex cheopis*. An explanation of this difference was obtained when it was found that *P. irritans* does not live well either on rats or on guinea-pigs. A count of the fleas was made daily in a number of experimental cages in which human fleas were living in company with wild Bombay rats. A great number of human fleas, caught in the manner we have already described, were put into a flea-proof cage along with a rat. Each day a census was made of the fleas still alive. After 24 hours it was found that only 1·2 per cent. of the fleas originally put in could be recovered, and after 72 hours this was further reduced to 1 per cent.

Another series of experiments, after the manner we have already described, was begun with the object of ascertaining how long human fleas remain infective, but as no fleas were ever found alive after the 5th day, this series had to be abandoned. However, each day a certain number of fleas were dissected and their stomach contents examined for the presence of plague bacilli. Table VIII contains the results of this examination. From this table it is seen that fleas with large numbers of plague bacilli in their stomach contents were found up to the 4th day, but that the proportion of infected fleas gradually diminished from the 2nd to the 4th day. It would appear, however,

from these results that multiplication of plague bacilli can take place in the stomach of the human flea.

TABLE VIII.

*Results of examination of human fleas (P. irritans) as to the presence of abundant plague bacilli in stomach contents after their being taken from septicaemic rats, the fleas in the meantime being fed on healthy animals.*

Interval between ingestion of septicaemic blood and examination of fleas	Number of fleas dissected	Number of fleas found with abundant plague bacilli in stomach contents	Percentage of fleas with abundant plague bacilli in stomach contents
1 day	2	1	50
2 days	9	5	55
3 „	41	11	27
4 „	14	2	14
5 „	3	0	0

(c) *Experiments with Ceratophyllus fasciatus.*

As these experiments were made at the end of the observations at Kasel and Dhand, and as this species of flea is obtained in very small numbers, only two complete experiments were carried out. The method used was exactly the same as that already described. The fleas were infected by allowing them to feed on septicaemic plague rats. On the death of the rats they were carefully examined with a lens so as to exclude any *P. cheopis*. They were then transferred to a healthy guinea-pig in a flea-proof cage.

Working in this way two experiments were made, with a successful result in each instance. In one case ten fleas were transferred. The guinea-pig died four days afterwards, and on post-mortem examination showed typical signs of plague, buboes being present in both inguinal regions. Pure cultures of *B. pestis* were obtained from the heart-blood. In the other instance four fleas were transferred. The guinea-pig died 12 days afterwards. Post-mortem examination revealed cervical buboes and other signs of plague. A pure culture of *B. pestis* was obtained from the heart-blood.

These two experiments show definitely that *Ceratophyllus fasciatus* can convey plague from infected to healthy animals.

*Summary.*

1. *Twenty-seven experiments to transmit plague from animal to animal by means of cat fleas (P. felis) were made. None of these was successful.*



2. *Thirty-eight experiments to transmit plague from animal to animal by means of human fleas (*P. irritans*) were made. Three were successful.*

3. *Two experiments made with *C. fasciatus* both gave successful results.*

V. QUESTION OF THE METHOD BY MEANS OF WHICH THE RAT FLEA (*P. cheopis*) TRANSMITS PLAGUE TO A HEALTHY ANIMAL.

We propose, in conclusion, to consider the question of how the flea transmits its infection to the healthy animal, on which problem some of the observations detailed above have a distinct bearing.

The following methods are possible:—

1. By the animal eating the infected fleas.
2. By the proboscis of the flea mechanically conveying the bacilli from the infected to the healthy animal.
3. By the salivary glands of the flea becoming infected, the bacilli being then inoculated along with the saliva.
4. By a regurgitation of the stomach contents through the oesophagus and pharynx, the bacilli being then injected with the saliva, or on the pricker, or being rubbed into the wounds made by the pricker.
5. By a retention of infected blood in the pharynx or about the mouth parts of the flea, the bacilli multiplying there and then being inoculated into the animal in the same manner as in No. 4 hypothesis.
6. By the bacilli contained in the faeces being deposited on the skin, and then being either injected by the pricker or rubbed into wounds made by the pricker.

Let us consider in detail the data bearing on each of these possible methods.

1. *By the Animal eating the infected Fleas.*

That this means of infection is of any importance, even if it may sometimes occur, is improbable for the following reasons:

1. Feeding experiments have shown that an animal is unlikely to become infected by the ingestion of material containing plague bacilli, unless the amount is considerable.

2. In 70 per cent. of the cases of infection by feeding, the animals developed a primary mesenteric bubo, but out of several hundred animals infected in the laboratory by means of fleas in no case was a mesenteric bubo discovered.

3. Infected fleas confined in test tubes readily convey the disease when allowed to bite an animal, in which case the situation of the primary bubo corresponds with the skin area upon which the fleas are placed.

2. *By the Proboscis of the Fleas mechanically conveying the Bacilli from the infected to the healthy animal.*

By this method of infection we mean that the proboscis of the flea acts merely as a mechanical instrument for transference of the bacilli from an infected to a healthy animal. It can be imagined that the external service of the flea's proboscis might become contaminated with plague bacilli while the flea was sucking the blood of a septicaemic rat, and that, on the flea transferring itself to a healthy animal, the proboscis might inject the bacilli under the skin.

It is impossible to exclude this means in the case of animals infected by fleas which have recently left a septicaemic host, but it is significant that the largest number of infections occurred with fleas which had fed upon infected animals within 36 hours. It is difficult, however, to suppose that contamination of the proboscis can explain those cases of continued infectivity for several days during which the insect fed regularly upon healthy animals.

Moreover, microscopical examination of the proboscis of more than 100 infected fleas failed to demonstrate the presence of plague bacilli.

3. *By the salivary glands of the Flea becoming infected, the Bacilli being then inoculated along with the Saliva.*

When it was ascertained that the rat flea could transmit infection from one animal to another, and especially when it was found that the flea might remain infective for several days, the possibility of a salivary gland infection suggested itself. We have carefully searched for the presence of plague bacilli in the body cavity and in the salivary glands of infected fleas. We have dissected and examined hundreds of fleas at different intervals after they had imbibed septicaemic blood, but on no occasion have we been able to find plague bacilli outside of the alimentary canal, and the salivary glands have always been found free from these organisms.

4. *By a regurgitation of the Stomach Contents through the Oesophagus and the Pharynx, the Bacilli being then injected with the Saliva or on the Pricker or being rubbed into the wounds made by the Pricker.*

We have seen above that the stomach of an infected flea may contain abundant plague bacilli. The hypothesis, which we have now to consider, is that the stomach contents may regurgitate through the pharynx, and that the plague bacilli contained therein may be injected along with the saliva or, soiling the pricker, may be injected by it, or being deposited on the surface of the skin may be rubbed into the pricker wounds.

In the paper on the physiological anatomy of the flea (vol. vi. p. 491) we have noted that the pharyngeal muscles contract like a wave from before backwards, the blood being passed into the stomach as a result of this wave-like action. On one or two occasions by means of a stereoscopic microscope we have been able to see this process taking place. We have no evidence that it may not be reversed, but it seems unlikely that a reverse action should take place.

The anterior end of the stomach is provided with an efficient valvular arrangement with the apparent object of preventing regurgitation, as shown by the following experiment, which has been tried several times. The stomach of a flea which had recently fed was dissected out intact. As long a portion of rectum as possible was left attached posteriorly. The oesophagus having been severed well in front of the valve, pressure was applied with a blunt instrument with the object of extruding the blood through the oesophagus. The posterior passage was closed by the contraction of the rectum. In no instance was it found possible to force the oesophageal passage, although sufficient pressure was applied to rupture the stomach.

On the other hand, in the case of fleas killed by immersion in alcohol, blood has been seen in the pharynx and mouth although the insects had not fed for some hours.

Plague bacilli have only rarely been seen in the oesophagus of plague-infected fleas, and when seen they have been few in number and only in fleas recently taken from plague-infected rats.

A study of the anatomy of the mouth parts of the flea, as described in our previous report, makes it clear that it is improbable that the saliva could become infected by matter regurgitated from the stomach. The regurgitated matter would escape by the mouth and

would then have to find its way into the salivary grooves in the mandibles. Another possibility is that the regurgitated matter might find its way into the aspiratory canal formed by the epipharynx and the mandibles and pass down that channel. We have no evidence bearing on this point. If regurgitation does take place we have no evidence bearing on the questions of the inoculation of the infected material mechanically by the pricker or its being rubbed in through the pricker wounds.

5. *By a retention of infected blood in the Pharynx or about the Mouth Parts of the Flea, the Bacilli multiplying there, and then being inoculated into the Animal in the same manner as in the last case.*

A study of the anatomy of the mouth parts of the flea suggests the possibility that plague bacilli, taken in with infected blood, might be retained in the pharynx or in the space between the epipharynx and hypopharynx and, multiplying there, be injected into the healthy animal in one of the methods we have mentioned above, but we have no evidence in favour of this possibility over and above what we have brought forward in the previous section.

We have microscopically examined the pharynx and mouth parts of many fleas taken from septicaemic rats but have never seen any plague bacilli in these situations.

In one or two instances we have seen blood in the pharynx and about the mouth of fleas which had not fed for some hours, and which had been killed by immersion in alcohol.

6. *By the Bacilli contained in the Faeces being deposited on the Skin and then being either injected by the Pricker or rubbed into the wounds made by the Pricker.*

As mentioned above, plague bacilli after being ingested into the stomach of a rat flea multiply there, and for many days after the flea has been taken from the infected rat, abundant plague bacilli can be demonstrated. We have also drawn attention to the fact that the flea while sucking has a habit of squirting blood *per anum*; that the faeces of infected fleas contain abundant plague bacilli, which are infective for guinea-pigs both when injected subcutaneously and when inoculated by the cutaneous method.

Experiments were made to ascertain if the pricks made by fleas were of sufficient size to allow plague bacilli to enter the body, no



other damage to the skin being done. Healthy fleas confined in a test tube were allowed to feed on an area of a guinea-pig's abdomen, the hair on which had been previously cut close without injuring the skin. Immediately afterwards a few drops of septicaemic blood of a rat which had died of plague, or of a virulent culture of *B. pestis*, were lightly spread over this area. The guinea-pig was then segregated. Many successful infections were obtained in this way. Similar experiments were made in which the plague culture was first spread on the skin and afterwards healthy fleas were allowed to feed on the same area. Successful infections were also obtained in this way.

The possibility of infection by the faeces in the manner above described has been demonstrated, but whether this is the usual process we have been unable to ascertain.

#### *General Summary and Conclusions.*

1. The average capacity of a rat flea's stomach is approximately 0.5 c.mm. On this basis a flea imbibing the blood of a plague rat showing a good septicaemia might take as many as 5000 germs into its stomach.

2. Multiplication of the plague bacillus takes place in the stomach of the rat flea.

3. The approximate proportion of fleas in the stomach of which multiplication of plague bacilli takes place has been determined, and it has been shown that this proportion varies with the season of the year, being six times greater in the epidemic season than in the non-epidemic season.

4. Plague bacilli are present in the rectum and faeces of fleas taken from plague rats, and such faeces are infective to guinea-pigs both by cutaneous and by subcutaneous inoculation.

5. On rare occasions plague bacilli have been found in the oesophagus, but never in any other region of the body, such as the body cavity or salivary glands.

6. During the plague season fleas might remain infective for 15 days after imbibing infective blood, but during the non-epidemic season no animal was infective after the 7th day.

7. A single rat flea may transmit the disease.

8. Both male and female rat fleas can transmit the infection.

9. Experimenting with cat fleas (*P. felis*) and human fleas



(*P. irritans*), 27 experiments with the former were unsuccessful, and out of 37 experiments with the latter three successes were obtained. Two experiments were made with *C. fuscatus*: both were successful. Multiplication of the plague bacillus takes place in the stomach of the human flea.

10. The plague bacillus has never been seen in the body cavity or in the salivary glands of infected fleas.

Evidence has been obtained to show that the bite of a healthy flea affords a sufficient avenue for infection by septicaemic blood if it is spread upon the bitten part.

No evidence has been obtained in favour of infection by contaminated mouth parts or regurgitation from the stomach, but the possibility of infection by such means cannot be excluded.

## XVI. EXPERIMENTAL PRODUCTION OF PLAGUE EPIDEMICS AMONG ANIMALS.

(*Second Communication.*)

In the first report (vol. VI. p. 450) we described several series of experiments which had as their object the determination of the relative importance of the Indian rat flea, *Pulex cheopis*, and of actual close contact in the absence of fleas in the dissemination of plague from animal to animal. These observations were carried out in a series of small godowns or cabins, which were built especially for the purpose. The experiments which we have now to put on record are a continuation of those already published. They were carried out during the non-plague season of 1906 and the plague season of 1907.

In the first paper we described in detail the structure of the godowns, and pointed out in what way they differ from one another. We need, therefore, now only recall to the reader's memory that the essential difference between them lies in the structure of the roofs. In the case of Nos. 1 and 2 the roofs, being of country tiles, offer good protection and shelter to the wild rat of Bombay; the flea supply in the interior is, therefore, abundant and regular. In the case of godowns 3 and 4 the roofs, being of flat Mangalore tiles, offer only poor protection to rats, and in consequence the flea supply is more or less scanty. As regards Nos. 5 and 6, it will be remembered that the roofs during the earlier experiments were made of corrugated iron fixed by cement to the tops of the walls. It was found impossible to keep these godowns absolutely free from fleas, and during one experiment, owing either to breeding or to a sudden migration from without through a flaw in the cement, a fairly abundant supply of these insects obtained access to godown No. 6.

In order to prevent any such unlooked-for circumstance happening again the corrugated iron was removed and a roof of "reinforced concrete" was put on each of these godowns before the present ex-

periments were begun. By this means we were able to keep Nos. 5 and 6 godowns absolutely free from fleas, and in consequence to obtain a number of clean contact experiments in which the presence of fleas was rigorously excluded.

#### SERIES A.

*Experiments in which epidemic plague did not occur when healthy guinea-pigs lived in close contact under conditions where access of fleas was completely prevented, but in which under otherwise similar conditions plague spread among the healthy animals in places where fleas were abundantly present.*

The same methods were adopted in this series of experiments as were used in the similar series already described in the first report. The godowns were not cleaned out during the whole period of the experiment, and the animals took their food from the floor which was contaminated with the excreta of their sick companions. Under these circumstances, as will be seen from the records below, in the case of godowns 5 and 6, which were free from fleas, no animals ever contracted plague, while in the case of Nos. 1 and 2 godowns, in which fleas were present, plague became epidemic, or not, according to the season of the year at which the experiment was made.

It will be convenient, and will conduce to the better understanding of the subject, if we describe the experiments in pairs, one in a flea-free godown, the other in a godown in which fleas were present. The experiments were always carried out contemporaneously in this way.

#### *Experiment I.*

This experiment was carried out in godowns Nos. 6 and 1 during the month of June, 1906, the beginning of the non-epidemic season in Bombay. The minimum temperature in the godowns was never below 80·5° F. and was frequently above 82° F.

*Godown 6.* On 5. VI. 06 there were placed in this godown 25 healthy guinea-pigs, and five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. By 11. VI. 06 all the inoculated guinea-pigs had died of plague. The uninoculated animals remained healthy until the experiment was ended on 2. VII. 06. No fleas were ever found on the guinea-pigs during the course of the experiment.

*Godown 1.* On 5. VI. 06 five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were placed in the

godown. A flea census on this day gave 39 fleas caught on five animals. All these guinea-pigs had died of plague by the 10. vi. 06. On 10. vi. 06, after the last inoculated guinea-pig had died, 25 healthy guinea-pigs were placed in the godown. On 21. vi. 06 one guinea-pig was found dead. It was proved not to be plague infected. All the other guinea-pigs remained healthy until the experiment was closed on 2. vii. 06. A flea census made on 19. vi. 06 showed 31 fleas on 25 guinea-pigs, and another count made on 1. vii. 06 gave 56 fleas on 24 guinea-pigs.

### *Experiment II.*

This experiment was carried out in godowns 6 and 1 during the month of July, 1906, that is, in the non-epidemic season in Bombay. The average mean temperature in the godowns during the period of the experiment was 82.3° F.

*Godown 6.* On 2. vii. 06 there were placed in this godown 25 healthy guinea-pigs and five others which had been inoculated with a virulent culture of *B. pestis*. By 6. vii. 06 all the inoculated guinea-pigs had died of plague. The uninoculated animals all remained well until the experiment was ended on 29. vii. 06. No fleas were ever found on the guinea-pigs during the experiment.

*Godown 1.* On 2. vii. 06 a preliminary flea count gave 50 fleas on 25 guinea-pigs. On this date there were put into the godown five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. The last of these animals died of plague on 6. vii. 06. On 6. vii. 06, after the last inoculated guinea-pig had died, there were placed in the godown 25 healthy guinea-pigs. Between 10. vii. 06 and 13. vii. 06 four of these healthy animals died, two from plague and two from some other cause. All the other guinea-pigs remained healthy until the experiment was abandoned on 27. vii. 06.

### *Experiment III.*

This experiment was carried out in godowns Nos. 6 and 2 during the month of August, 1906, when plague was not epidemic in Bombay, but at a season of the year when a slight recrudescence has been observed in former years. The mean temperature in the godowns during the period of the experiment was 81.1° F., while the minimum remained about 80° F.

*Godown 6.* On 1. viii. 06 there were placed in this godown 25 healthy guinea-pigs and five which had been inoculated with a

virulent culture of *B. pestis*. The first inoculated animal died on 6. VIII. 06 and the last on 12. VIII. 06. The uninoculated animals remained healthy until the experiment was abandoned on 5. IX. 06. No fleas were taken on the guinea-pigs during the course of the experiment.

*Godown 2.* On 1. VIII. 06, 126 fleas were taken on 25 guinea-pigs in the godown. The fleas were returned to the godown. On 1. VIII. 06 there were placed in the godown five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. By 8. VIII. 06 all these guinea-pigs had died of plague. On 8. VIII. 06, after the last inoculated animal had died, 25 healthy guinea-pigs were placed in the godown. On 15. VIII. 06 the first uninoculated guinea-pig died, and by 4. IX. 06 twenty-four out of the twenty-five had died, all from plague. On 5. IX. 06 on the surviving guinea-pig which appeared to be healthy 88 fleas were taken. The animal was then isolated, but remained healthy.

#### *Experiment IV.*

This experiment was carried out in godowns 6 and 2 during the months of September and October, when plague was still non-epidemic in Bombay. The mean temperature in the godowns during the period of the experiment was 81.8° F.

*Godown 6.* On 21. IX. 06 there were placed in this godown 25 healthy guinea-pigs and five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. By 29. IX. 06 all the inoculated guinea-pigs had died of plague. After this date fresh plague-inoculated guinea-pigs were added to the godown almost daily, the same number of infected animals being put in as died of plague in godown 2 during the course of the present experiment. Thus, between 2. x. 06 and 16. x. 06, twenty-three plague-infected guinea-pigs were added. All these animals died of plague, the last on 19. x. 06. It will be seen, therefore, that between 21. IX. 06 and 19. x. 06 the twenty-five healthy guinea-pigs were living in close contact with plague-infected animals. On 19. x. 06 one of the 25 uninoculated guinea-pigs was found dead. It was proved not to have died from plague. The remaining 24 animals remained healthy until the experiment was abandoned on 30. x. 06. No fleas were taken on any of the guinea-pigs during the course of the experiment.

*Godown 2.* On 21. IX. 06 there were placed in this godown five guinea-pigs which had been inoculated with a virulent culture



of *B. pestis*. All these guinea-pigs died of plague, the last on 27. ix. 06. Sixteen fleas which were taken from this animal were returned to the godown. On 27. ix. 06, after the last inoculated guinea-pig had died, 25 healthy guinea-pigs were put into the godown. On 1. x. 06 five of these animals were found dead, and by 16. x. 06 there only remained two alive. All the deaths were proved to be due to plague. On 17. x. 06 it was noticed that the two survivors had submaxillary buboes. On 30. x. 06 they were seen to be well. On them 101 fleas were taken. The guinea-pigs remained well until the experiment was abandoned on 30. x. 06.

*Experiment V.*

This experiment was carried out in godowns 5 and 1 during October and November, 1906, at a season of the year when plague was still non-epidemic in Bombay, but when the rat epizootic was just beginning. The mean temperature in the godowns during the period of the experiment was 80·6° F.

*Godown 5.* On 18. x. 06 there were placed in this godown 25 healthy guinea-pigs and five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. By 23. x. 06 all the inoculated guinea-pigs had died of plague. From this date onwards, as in the previous experiment, there were daily added to the godown more plague-infected animals corresponding to the number which died in godown 1. Thus between 24. x. 06 and 10. xi. 06 twenty-three plague-infected guinea-pigs were added, all of which died of plague, the last on 15. xi. 06. On some days the "concentration" of infection was very great. Thus, on 28. x. 06 the 25 healthy animals were living in contact with 19 guinea-pigs which were plague-infected. In spite of being thus exposed to severe contact infection for a period of about a month, all the 25 uninoculated guinea-pigs remained healthy until the experiment was abandoned on 28. xi. 06. No fleas were taken on any of these guinea-pigs during the course of the experiment.

*Godown 1.* On 18. x. 06 four guinea-pigs, which had been in the godown overnight, yielded 546 fleas. On 18. x. 06 there were placed in the godown five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. All these animals died of plague, the last on 21. x. 06. On 21. x. 06, after the last inoculated guinea-pig had died, 25 healthy guinea-pigs were put into the godown. Plague at once broke out amongst these animals, the first dying on 23. x. 06, and by 10. xi. 06 twenty-three of them had succumbed to the disease.

On 20. XI. 06 the two animals which remained were examined for fleas, and 416 were taken. They remained healthy till they were returned to stock on 3. XII. 06. The 416 fleas were put on a fresh guinea-pig in a flea-proof cage in the laboratory. This guinea-pig also remained healthy. It is evident, therefore, that although fleas were abundant the infection had died out.

#### *Experiment VI.*

This experiment was made in godowns 5 and 2 during the month of January, 1907, that is to say, at a season when the plague epizootic in Bombay was rapidly increasing. The mean temperature in the godowns during the period of the experiment was 72·8° F., and the maximum was never above 78° F.

*Godown 5.* On 8. I. 07 there were placed in this godown 25 healthy guinea-pigs and five guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. By 11. I. 07 all the inoculated animals had died of plague. After this date, as in the two previous experiments, more plague-infected guinea-pigs were added daily, 25 in all being put in between 15. I. 07 and 24. I. 07. All these guinea-pigs died of plague, the last death being on 31. I. 07. It is to be noted that on 18. I. 07 there were 21 plague-infected animals in contact with the 25 uninoculated guinea-pigs. These latter, however, remained healthy until the experiment was abandoned on 21. II. 07. No fleas were observed on the guinea-pigs throughout the course of the experiment.

*Godown 2.* On 8. I. 07 a preliminary flea count yielded 107 fleas on three guinea-pigs. On 8. I. 07 five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were placed in the godown. By 11. I. 07 all these animals had died from plague. On 11. I. 07, after the last inoculated guinea-pig had died, 25 healthy guinea-pigs were put into the godown. Plague at once broke out amongst these animals, the first dying on 15. I. 07, and in a fortnight from their being placed in the godown the last one had succumbed to the disease.

#### *Summary and Conclusions.*

In six contact experiments, carried out at different seasons of the year in godowns which were absolutely free from fleas, not a single healthy guinea-pig died of plague. In three of these experiments the contact with infected animals was kept up by the daily addition

of plague-infected animals for a period of from 14 to 21 days, and still none of the healthy animals developed plague. As the godowns were never cleaned out, close contact includes contact with faeces and urine of infected animals, and eating of food contaminated with faeces and urine of infected animals. We can, therefore, conclude that close contact of infected animals with healthy animals, if fleas are excluded, does not give rise to an epizootic amongst the latter.

At the same time as these flea-free contact experiments were being carried out, experiments in similar godowns, but which received a regular supply of fleas from the roofs, were made. The details of these experiments are summarised in the following table. From this table it is seen that the experiment in June failed completely, that the one in July nearly completely failed, and that the four experiments made between August and January were successful. We can conclude that fleas, and fleas only, were responsible for these epizootics. In the January

TABLE.

No. of Exp.	Go- down	Season of year	Average daily mean temperature of godown	Flea census	No. of guinea- pigs exposed to infection	No. of guinea-pigs which died from plague					No. of days from first exposure to infection to death of last guinea-pig
						1st week	2nd week	3rd week	4th week	Total	
I	1	June 1906	Min. temp. never below 80.5° F. and often above 82° F.	39 on 5 guinea-pigs	25	0	0	0	0	0	—
II	1	July 1906	82.3° F.	50 on 25 guinea-pigs	25	1	1	0	0	2	8
III	2	August 1906	81.1° F.	126 on 25 guinea-pigs	25	0	14	8	2	24	28
IV	2	Sept. & Oct. 1906	81.8° F.	101 on the last two guinea-pigs	25	7	13	3	0	23	20
V	1	Oct. & Nov. 1906	80.6° F.	546 on 4 guinea-pigs	25	17	4	2	0	23	21
VI	2	January 1907	72.8° F.	107 on 3 guinea-pigs	25	14	11	0	0	25	14

experiment, that is to say, at a season of the year when the rat epizootic in Bombay city is rapidly rising, the epizootic among the godown guinea-pigs ran a very rapid course, all the animals succumbing to the disease in a comparatively short time. On the other hand, in the three positive experiments during the non-epizootic season in Bombay, in every instance, and especially in August, the epizootic

among the godown guinea-pigs was more or less drawn out, and one or two of the animals escaped, the infection dying out towards the end.

The bearing of these facts on the seasonal prevalence of plague will be dealt with in another paper, when the factors of the flea prevalence and climatic conditions in relation to plague epidemics will be fully discussed.

#### SERIES B.

*Experiments indicating that the number of fleas in the godowns affects the rate of progress of the epizootic amongst the guinea-pigs.*

In the previous report we brought forward evidence which indicated that if fleas are present, then the epizootic once started spreads from animal to animal, the rate of progress being directly influenced by the number of fleas present. We pointed out that in the experiments during the months of December, January, and February the progress of the epizootic was very rapid in those godowns, namely, Nos. 1 and 2, in which the flea population was abundant and was kept up by a natural supply from the roof; that it was much slower in godown No. 5 in which the flea supply was kept up artificially; and finally that it was slowest of all in godown No. 6 in which there was no definite natural supply of fleas, and from which the fleas were daily removed for a period of six days, after which removal only a comparatively small number could be caught.

We have now to detail two observations which were made in godowns 3 and 4 which are roofed with Mangalore tiles, and in which the flea infestation has never been so great as in Nos. 1 and 2 (*vide* p. 453 of last report). We shall contrast the rate of progress of the epizootics in these godowns with its rate in godowns 1 and 2 at the same season of the year, that is to say, when the climatic conditions were suitable.

#### *Experiment VII.*

*Godown 4.* On 5. XII. 05 twenty healthy guinea-pigs were placed in this godown. On 6. XII. 05 a flea census was made and 35 fleas were taken on 12 animals. Between this date and 13. XII. 05 somehow or other plague infection got into the godown, the first guinea-pig dying on 13. XII. 05. The epizootic once started went slowly through the rest of the guinea-pigs, the last animal dying on 15. I. 06, that is to say, it took 33 days to kill all the 20 guinea-pigs. This experiment



can be contrasted with an experiment which was made in godown 1 at the same time (Experiment V, p. 457 of previous report). In this instance only seven days elapsed between the death of the first and last guinea-pigs, although the epizootic had to pass through 26 instead of 20 animals. In this case the fleas were present in very large numbers, 115 being taken on the last five animals when moribund.

*Experiment VIII.*

*Godowns 4 and 2.* This experiment was carried out in godowns 4 and 2 during the rise and height of the plague epizootic of 1907.

*Godown 4.* On 29. I. 07 a flea census yielded nine fleas on three guinea-pigs. On 30. I. 07, 100 fleas were added. On 1. II. 07 five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were put into the godown. The first of these animals died on 3. II. 07, and the last on 5. II. 07. On 5. II. 07, after the last inoculated guinea-pig had died, 25 healthy guinea-pigs were allowed to run about free in the godown. The course and rate of progress of the epizootic which broke out amongst these animals are well shown in the accompanying chart (p. 430). The first animal died on 12. II. 07 and after that date the epizootic progressed extremely slow, the last guinea-pig dying on 14. IV. 07. From the date of the exposure of the guinea-pigs to infection until the death of this last animal there was therefore an interval of 68 days. A census of the fleas in the godown was made on several occasions during the course of the experiment. The results of these counts are recorded on the chart. It will be seen that except at the end of the experiment, when only the last animal remained alive, the fleas were at no time numerous. The large increase towards the end was probably due to breeding, which for some reason or other suddenly started. In great contrast with the epizootic of slow progress in this godown is the epizootic which took place in godown 2, while the former was in progress.

*Godown 2.* On 1. III. 07 a census yielded 1246 fleas caught on one guinea-pig. On 1. III. 07 five guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were put into the godown. The first of these animals died on 5. III. 07 and the last on 7. III. 07. On 6. III. 07, while there was still one of the inoculated guinea-pigs alive, 25 healthy guinea-pigs were allowed to run free in the godown. On 9. III. 07 the first uninoculated guinea-pig died; on 10. III. 07 sixteen died; on 11. III. 07 four; on 12. III. 07 four;



all of these animals were proved to be plague-infected. On 12. III. 07, that is to say, six days after the guinea-pigs had been exposed to infection, only one remained alive. This guinea-pig was noticed to be sick and to have a bubo in the cervical region. On 15. III. 07,

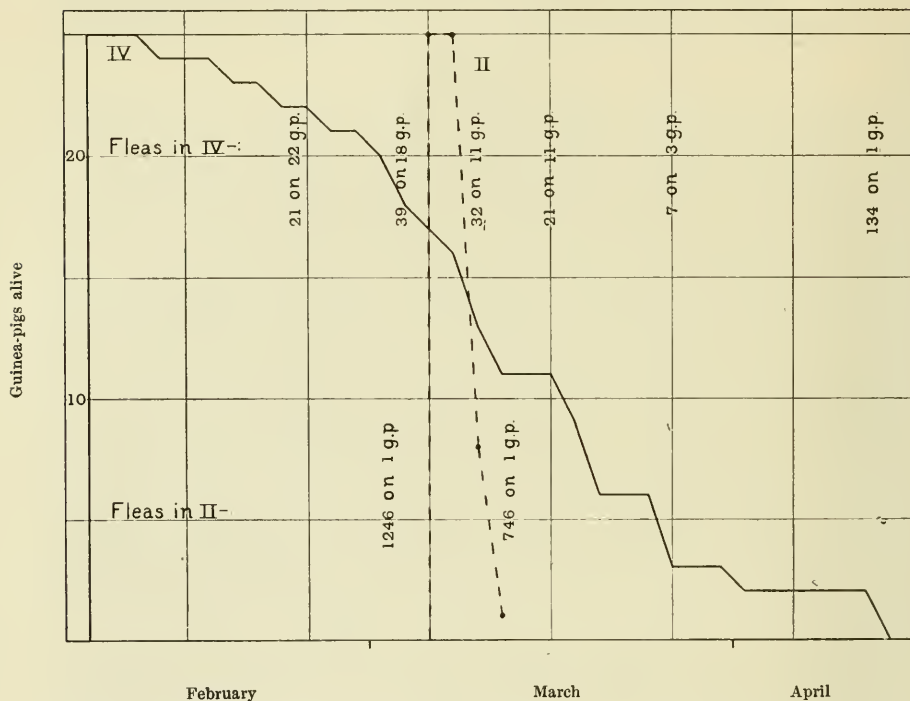


Chart showing the rate of progress of the epizootics which occurred at the same season of the year, one in godown 4 (black line) and the other in godown 2 (broken line). The curves are constructed on the number of guinea-pigs left alive in the godowns at intervals of two days.

as it appeared to be recovering, it was killed with chloroform and examined bacteriologically. A pure culture of *B. pestis* was obtained. The rate of progress of this epizootic is shown as a broken line on the chart. Its great rapidity as contrasted with the slowness of the epizootic in No. 4 godown is very remarkable. As both experiments were made at the same season of the year and under the same conditions, except as regards the number of fleas present, we can only conclude that this latter factor was the one which determined the difference between the two epizootics.

*Summary and Conclusions.*

Two experiments are cited, in each of which the plague epizootic amongst the guinea-pigs in a godown, which was only slightly infested with fleas, is contrasted, as regards its rate of progress, with that amongst the guinea-pigs in a godown which was abundantly supplied with fleas. In each instance the rate of progress is shown to be much slower in the former than in the latter godown. We have, therefore, confirmation of our previous conclusion that, if fleas are present, then the epizootic once started spreads from animal to animal, the rate of progress being conditioned by the number of fleas present. The question of the influence of a flea prevalence on the seasonal prevalence of plague will be dealt with in another paper.

SERIES C.

*Experiment indicating that, when an epidemic has occurred amongst a number of guinea-pigs, the contagion still remains in the place, and is effective in proportion as the test animals are accessible to, and found to be infested with, fleas.*

The single experiment which makes up this series is a confirmation of Experiments VII and IX of Series C described in the last report.

*Experiment IX.*

On 28. 1. 07 into godown 2, in which a plague epizootic had just come to an end by killing all the guinea-pigs, three groups of four guinea-pigs each were placed, namely,

- (A) Four guinea-pigs running about free on the floor;
- (B) Four guinea-pigs in wire cages two inches above the ground; and
- (C) Four guinea-pigs in wire cages suspended two feet above the ground.

On 30. 1. 07 these guinea-pigs were freed from fleas under chloroform, removed from the godown, and segregated. 125 fleas were caught on the four guinea-pigs which had been running about, and 27 on the four which had been placed in cages two inches from the ground. No fleas were taken on the animals which had been suspended two feet above the ground. The eight animals of groups A and B were observed to be ill and all to have buboes in the cervical region. Three showed typical phlyctenules under their chins. The

four guinea-pigs of group *C* appeared quite well. The fate of the animals of these three groups was as follows:—

Group *A*. One died under chloroform while the fleas were being removed. It was proved bacteriologically to be plague infected, the bubo being in the cervical region. The other three guinea-pigs all died of plague, one on 31. I. 07, one on 1. II. 07, and the third on 2. II. 07. The buboes were all cervical.

Group *B*. All four animals died of plague, three on 31. I. 07, and the fourth on 2. II. 07. The bubo was in every instance in the cervical region.

Group *C*. All four guinea-pigs remained healthy.

#### *Conclusions.*

Infection can therefore take place without any contact with contaminated soil, as the guinea-pigs which were kept two inches above the ground all contracted the disease. Aerial infection is also excluded, since the guinea-pigs which were suspended two feet from the ground remained healthy, while those running free and those two inches from the ground became infected.

We conclude that infected fleas were the agents of transmission in this experiment.

#### SERIES D.

*Experiments in which monkeys, protected and unprotected from fleas, were exposed in an infected godown. The protected monkeys never developed plague, while a certain number of those left unprotected were attacked.*

The cages which were used in this series of experiments were of the same pattern as those employed in our experiments in plague-infected houses in Bombay. They have already been described and figured (*vide* Plates VI and VII, p. 476 of first report). The essential feature was that in one cage protection from fleas was obtained by means of a layer of fine-meshed metallic gauze, which was absent from the unprotected cages.

#### *Experiment X.*

*Godown 1.* On 23. XI. 06 a preliminary flea count yielded 682 fleas on three guinea-pigs. On this day three guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were placed in the

godown. These three guinea-pigs had all died of plague by 30. XI. 06. On 30. XI. 06, after the death of the last guinea-pig, four monkeys were put into the godown, two protected, and two unprotected from fleas. On 2. XII. 06 the cages were removed, the monkeys freed from fleas under chloroform, and isolated. No fleas were got on the monkeys which had been in the wire gauze cages, while 16 fleas were got on one of the unprotected animals and 48 on the other. Both the protected monkeys remained well, while both the unprotected died of plague, the one on which the greater number of fleas was taken dying five days before the other. The diagnosis was bacteriologically confirmed in each instance. On 3. XII. 06, that is the day after the four monkeys were removed from the godown, without any fresh infection being introduced, two monkeys in ordinary open cages were put into the godown. On 5. XII. 06 the monkeys were removed, cleared of fleas under chloroform, and isolated. On one animal 23 fleas and on the other 33 fleas were obtained. On 8. XII. 06 the monkey which had the greatest number of fleas on it died of plague. The other monkey remained healthy.

#### *Experiment XI.*

*Godown 1.* On 9. XII. 06 a preliminary flea count gave 313 fleas on three guinea-pigs. On 10. XII. 06 there were placed in the godown three guinea-pigs which had been inoculated with a virulent culture of *B. pestis*. The last of these animals died on 14. XII. 06. On 14. XII. 06 four monkeys were placed in the godown, two in wire gauze cages, and two in cages which left them unprotected from fleas. On 16. XII. 06 the cages were removed, the monkeys freed from fleas under chloroform, and then isolated. On the protected monkeys no fleas were obtained, while five fleas on one and 21 fleas on the other of the unprotected animals were taken. All four animals remained healthy.

#### *Experiment XII.*

*Godown 1.* On 12. I. 07 a preliminary flea count yielded 194 fleas on six guinea-pigs. On this date three guinea-pigs, which had been inoculated with a virulent culture of *B. pestis*, were placed in the godown. By 17. I. 07 all these guinea-pigs had died from plague. On 17. I. 07 four monkeys were placed in the godown, two in wire gauze cages, and two in cages which gave access to fleas. On 19. I. 07 the cages were removed, the monkeys freed from fleas under chloroform,

and then isolated. On the protected monkeys no fleas were obtained, while 18 fleas on one and 24 fleas on the other of the unprotected animals were taken. All four monkeys remained healthy.

#### *Experiment XIII.*

*Godown 1.* On 19. I. 07 three guinea-pigs which had been inoculated with a virulent culture of *B. pestis* were placed in the godown. On 20. I. 07 a flea census yielded 271 fleas on these animals. They had all died of plague by 23. I. 07. On 22. I. 07, the last inoculated guinea-pig being still alive, four monkeys were placed in the godown, two in wire gauze cages, and two in cages which did not give protection from fleas. On 25. I. 07 the cages were removed, the monkeys freed from fleas, and isolated. On the protected animals no fleas were obtained, while 19 fleas from one and two fleas from the other of the unprotected monkeys were taken. Both the protected animals remained well. The monkey on which 19 fleas were obtained died of plague on 27. I. 07. The monkey on which two fleas were got remained healthy.

#### *Experiment XIV.*

*Godown 2.* On 2. II. 07, when an epizootic amongst a number of guinea-pigs was just coming to an end in this godown, the last guinea-pig not having yet died, four monkeys were placed in the godown, two in wire gauze cages, and two in cages which did not give protection from fleas. On 4. II. 07 the guinea-pig died, and 36 fleas taken from its corpse were returned to the godown. On 5. II. 07 the monkeys were removed from the godown, freed from fleas, and segregated. On the protected animals no fleas were found, while seven fleas from one and two fleas from the other of the unprotected monkeys were taken. Both the protected animals remained well. The monkey on which two fleas were obtained died of plague on 8. II. 07. The other monkey remained healthy.

#### *Conclusions.*

Five experiments with 22 monkeys were made; in 10 cases they were protected from fleas and in 12 were unprotected. None of the protected animals contracted the disease, while five out of the 12 unprotected monkeys died of plague.

Soil infection was excluded, and all the monkeys were equally exposed to aerial infection.



We must conclude, therefore, that infected fleas in the godown were alone responsible for the infection of the five unprotected animals which died of plague.

*Summary and Conclusions.*

1. Close and continuous contact of plague-infected animals with healthy animals, if fleas are excluded, does not give rise to an epizootic among the latter. As the godowns were never cleaned out, close contact includes contact with faeces and urine, and eating of food contaminated with faeces and urine of infected animals.

2. When fleas are present, the epizootic, if it does start, varies in severity and rate of progress according to the season of the year and the number of fleas present. The season in which epizootics were readily produced experimentally, and spread rapidly, corresponds with that of the plague epidemic.

3. An epizootic of plague may occur in a godown containing infected fleas without direct contact of healthy animals and infected animals (Experiments III, IV, V, and VI).

4. In an infected godown the infection is effective in proportion as the test animals are accessible to fleas.

5. Infection can take place without any contact with contaminated soil (Experiments IX to XIV).

6. The experiments exclude aerial infection.

7. The experiments lead to the conclusion that fleas, and fleas alone, were the transmitting agents of infection.

## XVII. EXPERIMENTS IN PLAGUE HOUSES IN BOMBAY.

*(Second Communication.)*

In a paper published in the first report (vol. VI. p. 467) we detailed a number of observations which were carried out in plague houses in Bombay and which went to prove that in a plague-infected house the infection is due to the presence therein of infected rat fleas, which were capable of transmitting the disease to animals.

During the epidemic of 1907 we have confirmed and amplified these previous observations.

### I. OBSERVATIONS IN CONFIRMATION OF THOSE MADE LAST PLAGUE SEASON.

The houses which were used for these observations were selected with a certain amount of discretion, our object being to ensure that they were really plague infected. In the majority of instances before using the houses for experimental purposes we satisfied ourselves (*a*) that there had been two or more cases of plague in the house, or (*b*) that there had been a single case of plague with a history of dead rats, or (*c*) that a dead rat had been found, but no plague case had occurred. In some instances at the beginning of the observations we were satisfied if a dead rat, which had been proved plague infected at the laboratory, had been found alongside the building in which a plague case had occurred, the presumption being that the rat had been thrown out from the house. It was soon found, however, that such houses seldom or never yielded successful results. They were, therefore, omitted in the later experiments.

The experiments, following the classification made in the first paper, may be conveniently divided into two groups.

GROUP I.

*Experiments with Guinea-pigs running free in plague houses.*

In the similar experiments of last year already detailed, out of 42 experiments twelve houses (29 %) proved infective for guinea-pigs which had been placed therein, the animals dying of plague within a few days after they had been removed from the house. In practically all these guinea-pigs the situation of the primary bubo was in the cervical region.

In the present series of experiments as a general rule only one guinea-pig was put into each house. It was left in for 18 hours. In all 100 houses were tested in this way. The guinea-pig died of plague in 19 instances, that is to say, 19 % of the houses yielded successful results. The details of the successful experiments are given in Table I. It is to be noted that the distribution of the primary bubo in these animals was as follows:—no bubo, 1: buboes in neck only, 15: buboes in neck and groin, 3: that is to say, in every case which had a bubo the neck glands were affected. The significance of this distribution of the primary bubo has already been dealt with in another paper (p. 382).

The question of the number of fleas taken in these houses will be discussed later on.

*Summary and Conclusions.*

In 19 out of 100 experiments guinea-pigs allowed to run free in houses which were deemed to be plague infected developed the disease and died.

GROUP II.

In this group of observations, which is made up of two series, fleas obtained in plague houses were fed on guinea-pigs in flea-proof cages in the laboratory. The two series only differ from one another in the manner in which the fleas were obtained.

SERIES I.

*Experiments with fleas caught on plague-infected rats found in houses.*

In the series of experiments of this nature, which have been already reported, on three occasions, namely, on every occasion on which the experiment was made, fleas transferred from plague-infected rats found



in houses in Bombay were able to transmit the disease to healthy animals in flea-proof cages in the laboratory.

In the present series of experiments, also three in number, an exactly similar result was obtained, that is to say, all the guinea-pigs, to which the fleas were transferred, died of plague. The details of these three experiments are given in Table II. In the first two experiments the rats were sent dead to the laboratory. There the fleas were removed and at once transferred to a guinea-pig, which in each instance died of plague in a few days. The history of the third experiment is instructive. In a commercial office in the Fort two dead rats were found in the corner of a large room. One of these rats was proved to be plague infected at the laboratory. That same evening a guinea-pig was allowed to run free in this corner, being shut off from the rest of the office by means of a low barricade. On visiting the office

TABLE II.

*Experiments with fleas caught on plague-infected rats found in houses.*

Serial No.	Date	Address	No. of fleas	Animal on which fleas were fed	Result	Position of primary bubo	Remarks
1	20. 2. 07	84, 1st Lane Kamathipura	13	Guinea-pig	Died of plague	Double submaxillary and cervical	Rat was found dead in the house.
2	21. 2. 07	5 Victoria Road	12	Guinea-pig	Died of plague	Left inguinal	Rat was found dead in stable.
3	19. 3. 07	29 Hummum St.	6	Guinea-pig	Died of plague	Double cervical, left sub-maxillary	Rat was found dead in an office at above address.

next morning we found a fresh dead rat lying on the floor outside the barricade underneath the desk of a clerk. On this rat, which was proved to be plague infected at the laboratory, six fleas were taken. These fleas were transferred to a fresh guinea-pig in the laboratory, which animal died of plague. Over the spot on which the rat was found a guinea-pig was allowed to run backwards and forwards for about one minute. It was then chloroformed and searched for fleas, 82 being captured on it. A second guinea-pig was allowed to run over this place for a few minutes: 32 fleas were caught on this second animal. Of these 114 fleas 29 were dissected: in the stomach contents of 17 abundant plague bacilli were found on microscopical examination. The remainder of the fleas were transferred to a fresh guinea-pig in the laboratory, which guinea-pig died of plague in four days. Neither of the guinea-pigs which had been used as traps to catch the fleas developed plague.



*Summary.*

On three occasions, namely, on every occasion on which the experiment was made, fleas transferred from plague-infected rats found in houses in Bombay were able to transmit the disease to healthy animals in flea-proof cages in the laboratory.

## SERIES 2.

*Experiments with fleas caught on guinea-pigs which had been left for some hours in plague houses.*

In this series of experiments the fleas caught on guinea-pigs, which had been left in plague houses running about free for about 18 hours, were transferred to fresh guinea-pigs in flea-proof cages in the laboratory.

In the series of experiments of this nature, which was made last year and which has been already reported (vol. VI. p. 475), in 8 out of 40 instances (20 %) the animals to which the fleas were transferred died of plague. The situation of the primary bubo in these animals was in the great majority of cases in the cervical region.

In the present series of experiments, fleas captured in the manner mentioned were transferred on 31 occasions to fresh guinea-pigs in the laboratory. 11 of these animals died of plague, namely, 35 % of successful transmissions. The details of these cases are given in Table III, from which it is seen that the distribution of the primary bubo was as follows:—neck alone 4 cases, groin alone 2 cases, neck and groin 4 cases, neck and axilla 1 case. Thus the neck glands were affected in 8 out of 11 instances.

*Summary.*

Rat fleas captured in plague houses are capable of giving plague to fresh guinea-pigs in the laboratory.

## II. OBSERVATIONS ON THE NUMBER OF RAT FLEAS CAUGHT IN PLAGUE AND NON-PLAGUE HOUSES IN BOMBAY DURING THE PLAGUE SEASON.

In the course of the observations made last year a count was made of the fleas caught on the guinea-pigs which had been allowed to run free in plague houses (vol. VI. p. 482). In one group of these houses disinfection with perchloride of mercury solution or with the fumes of

TABLE III.

*Experiments with fleas caught on Guinea-pigs which had been left for some hours in plague houses.*

Serial No.	Date	Address	No. of fleas	Animal on which fleas were fed	Result	Position of primary bubo	Remarks
1	30. 1. 07	13 Girgaum Road	30	Guinea-pig	Died of plague	Right submaxillary and cervical	2 plague cases: a sick rat had been killed.
2	7. 2. 07	385 Girgaum Road	38	"	"	"	3 plague cases in house, 3 dead rats had been found in the room from which the fleas were got.
3	31. 1. 07	B. Bacteriological Laboratory	20	"	"	Double submaxillary and cervical, right axillary	A dead plague-infected rat had been found in this room.
4	27. 2. 07	5 Victoria Road	13	"	"	Right submaxillary and cervical	2 dead rats proved plague infected had been found in this room.
5	27. 2. 07	5 Victoria Road	17	"	"	Double submaxillary and cervical	1 dead rat proved plague infected had been found in this room.
6	1. 3. 07	43 Moreland Road	55	"	"	Right submaxillary and cervical, left inguinal	1 plague case: 2 dead rats had been found in the room.
7	12. 3. 07	29 Hummum Street	29	"	"	Double inguinal	2 dead rats had been found in the corner of this office where the guinea-pig was allowed to run free.
8	13. 3. 07	29 Hummum Street	82	"	"	Double submaxillary and cervical, double inguinal	1 dead rat had been found and the fleas were got from 2 guinea-pigs which were left in this spot for a few minutes.
9	25. 3. 07	Superintendent's Bungalow, Colaba Asylum	35	"	"	Double inguinal	1 dead rat proved plague infected and 1 not examined found in room.
10	1. 4. 07	Colaba Asylum (2nd room), Clerk's House	20	"	"	Double submaxillary and right inguinal	2 putrid rats found in this room on 1. 4. 07.
11	1. 4. 07	Colaba Asylum, Clerk's House	55	"	"	Right cervical and left inguinal	1 dead rat found on 1. 4. 07.

SO<sub>2</sub> had been carried out just before the guinea-pigs were put in. In the case of each of these two groups we can divide the houses into two classes, namely, (*a*) a class in which the houses were definitely proved by us to be plague infected, either because plague-infected rats had been found in the house or because one or both of the guinea-pigs developed the disease, and (*b*) a class, made up of the remainder, in which the houses were only presumably plague infected, inasmuch as one or more plague cases had occurred in them. The data concerning the flea census in these two classes of houses are summarised in Table IV. From this table it is seen that the average number of fleas in the houses, which were definitely proved to be plague infected, was about three times greater than in the houses which were only presumably plague infected. The average number of fleas was much larger in the disinfected than in the non-disinfected houses. This was probably due to a few very large counts, rather than to an increase distributed equally over the majority of the counts.

TABLE IV.

*Showing data referring to flea census in plague houses in the epidemic of 1906.*

	Houses not previously disinfected			Houses previously disinfected		
	No. of houses examined	Total no. of fleas caught	Average no. of fleas per house	No. of houses examined	Total no. of fleas caught	Average no. of fleas per house
Houses definitely proved } plague infected	13	445	34	10	731	73
Houses only presumably } plague infected	29	388	13	21	488	23

During the plague season of 1907 we have obtained further data which show that the average number of fleas in houses which can be definitely proved to be plague infected is greater than in houses which are only presumably infected, and greater in the latter than in houses in which neither plague cases had occurred nor dead rats been found.

The method which was adopted to obtain these data was as follows:—

On being advised of a plague-infected house, which on examination proved satisfactory, a guinea-pig was allowed to run free in it. At the same time a house, in which no plague case had occurred nor dead rats been found, was selected in the same building or in a building of the same type in the immediate neighbourhood. In this house another guinea-pig was set free.

As many houses as obtainable were treated each evening in this way. Next morning the guinea-pigs were chloroformed and searched for fleas. They were then brought back to the laboratory and segregated. The fleas which were caught were in some instances dissected, the contents of the stomach being examined as to the presence or absence of plague bacilli, and in other instances transferred to a fresh guinea-pig in a flea-proof cage. If the number of fleas was large some were dissected and the remainder transferred.

The houses operated on were classified into groups as before.

*Group A.* Houses deemed plague infected for one or more of the following reasons:—1. A dead plague-infected rat or rats had been found in the house. 2. The guinea-pig which was allowed to run free in the house developed plague and died. 3. Some of the fleas caught on the guinea-pig which was allowed to run free in the house were found on dissection to be plague infected. 4. Fleas caught on the guinea-pig which was allowed to run free in the house gave plague to a healthy guinea-pig, to which they were subsequently transferred.

*Group B.* Houses presumably plague infected (1) either, because a plague case or cases had occurred in the house, (2) or, because dead rats, which had not been sent for examination, were found in the house.

*Group C.* Control houses of same type and situated in same neighbourhood.

The results of the flea census in these three groups of houses are set forth in Table V.

TABLE V.

*Showing data referring to the flea census in houses classified according as to whether they were plague infected or not.*

	Total no. of houses	Total no. of fleas taken	Average no. of fleas taken per house
Group A—namely, houses proved to be plague infected ... .. }	27	784	29
Group B—namely, houses not proved to be, but presumably, plague infected }	73	755	10·3
Group C—namely, houses which were not plague infected ... .. }	68	169	2·5

Houses which were definitely proved to be plague infected contained on an average nearly three times the number of rat fleas contained in houses which were only presumably plague infected and 12 times the number contained in houses which were free from suspicion. The details

TABLE VI.

*Details of experiments made in houses which were proved plague infected.*

Serial No.	Address	No. of fleas taken	Reasons for classifying as plague infected
1	13 Girgaum Road	40	Running free guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
2	385 Girgaum Road (Bedroom)	10	Running free guinea-pig died of plague. 6 fleas dissected, 4 found plague infected.
3	385 Girgaum Road (Kitchen)	38	Running free guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
4	Office in Bombay Bact. Laboratory	20	Dead plague-infected rat found. Guinea-pig to which fleas were transferred died of plague.
5	82--84, 14th Lane, Kama-thipura	18	Dead plague-infected rat found. 16 fleas dissected, 9 found plague infected.
6	159 Queen's Road	11	11 fleas dissected, 2 found plague infected.
7	New Sewri Road	18	Running about guinea-pig died of plague.
8	Shop in Esuf building	12	Running about guinea-pig died of plague.
9	Godown, 5 Victoria Road	13	Dead plague-infected rat found. Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
10	Godown, 5 Victoria Road	17	Dead plague-infected rat found. Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
11	43 Morland Road	55	Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
12	9 Military Square	8	8 fleas dissected, 1 found plague infected.
13	5 Victoria Road	1	Running about guinea-pig died of plague.
14	199 Clerk Road	17	Running about guinea-pig died of plague.
15	29 Hummum Street	29	Dead plague-infected rat found. Guinea-pig to which fleas were transferred died of plague.
16	29 Hummum Street	114	Dead plague-infected rat found. Guinea-pig to which fleas were transferred died of plague. 29 fleas dissected, 17 found plague infected.
17	65 Sonapur Street	66	Running about guinea-pig died of plague.
18	87 Fanaswadi	5	4 fleas dissected, 3 found plague infected.
19	236 Doctor Street	20	Running about guinea-pig died of plague. 20 fleas dissected, 1 found plague infected.
20	104 Sonapur Street	137	Running about guinea-pig died of plague.
21	Asylum, Colaba, Superintendent's Bungalow	3	Running about guinea-pig died of plague. 3 fleas dissected, 2 found plague infected.
22	Asylum, Colaba, Superintendent's Bungalow	35	Dead plague-infected rat found. Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
23	Godown, Jubilee Mills, Sewri	6	Running about guinea-pig died of plague.
24	1 Dadysett Agiary	14	14 fleas dissected, 1 found with abundant <i>B. pestis</i> in stomach contents. Running about guinea-pig died of plague.
25	Havildar's House, Lunatic Asylum, Colaba	2	One dead rat proved plague infected found in room.
26	Clerk's House, Lunatic Asylum, Colaba	55	Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.
27	Lunatic Asylum, Colaba Clerk's House (2nd room)	20	Running about guinea-pig died of plague. Guinea-pig to which fleas were transferred died of plague.



of the experiments in the 27 houses which were proved to be plague infected are set forth in Table VI.

A further point of interest in these observations is the proportion of fleas with plague bacilli in their stomach contents. In all 130 fleas were dissected and of these 41, or 32 %, contained in their stomachs bacilli microscopically indistinguishable from *B. pestis*. It is also worthy of mention that in all the 27 houses which were proved to be plague infected, with the exception of three, namely Nos. 8, 17 and 24, dead rats had been found shortly before the experiments were made.

*Summary.*

During the plague epidemic season of 1907 in Bombay a census of rat fleas in houses classified as follows has been made:—*A*, houses definitely proved to be plague infected: *B*, houses which were presumably, but not proved to be, plague infected: *C*, control houses.

In the case of group *A* the average number of fleas taken was three times greater than in the case of group *B* and 12 times greater than in the case of group *C*.

*General Summary and Conclusions.*

1. In 19 out of 100 experiments guinea-pigs allowed to run free in houses which were presumably plague infected developed the disease and died.

2. On three occasions, namely, on every occasion on which the experiment was made, fleas transferred from plague-infected rats found in houses infected fresh guinea-pigs in the laboratory.

3. Rat fleas, caught on guinea-pigs in plague houses and transferred to fresh guinea-pigs, transmitted the disease in 35 % of the cases.

4. A census of rat fleas in houses in Bombay, which were proved plague infected, indicated that rat fleas were 12 times as numerous as in control houses; and that in presumably infected houses rat fleas were four times as numerous as in control houses.

5. In 41 out of 130 fleas taken on guinea-pigs in plague-infected houses, bacilli microscopically indistinguishable from plague were found in the stomach.

6. In the case of 24 of the 27 houses definitely proved to be plague infected, dead rats had been found shortly before the experiments were made.

XVIII. ON THE EXTERNAL ANATOMY OF THE INDIAN  
RAT FLEA (*P. CHEOPIS*), AND ITS DIFFERENTIATION  
FROM SOME OTHER COMMON FLEAS.

(Plates X to XII.)

In a previous paper (vol. VI. p. 486) the physiological anatomy of the mouth parts and alimentary canal of *P. cheopis* has been described. In the present paper we propose to give a detailed description of the external morphology.

For those who are not familiar with entomological nomenclature it is necessary briefly to detail the general structure of a flea.

GENERAL STRUCTURE OF A FLEA.

A flea has its body laterally flattened and presents for description head, thorax and abdomen.

*Head.* The shape of the head varies considerably in different species. It is firmly united behind to the thorax. Attached to the head antero-ventrally will be found the mouth parts. In some species of flea, *P. felis* for example, a row of large dark, peg-like teeth or bristles is found around the antero-ventral margin of the head. We shall call these bristles the *perioral comb of bristles* (Plate XII).

A deep groove will be noted on the antero-lateral surface of the head with its long axis lying obliquely downwards and backwards. In this groove (antennary pit), which divides the lateral surface of the head into two portions, the antenna lies (Plate X).

An eye (in those species which possess this organ) is situated on the anterior margin of the antennary groove (Plate X).

A number of bristles are found over the surface of the head, the number and arrangement of these bristles being useful in distinguishing the species.

*Thorax.* The thorax consists of three segments jointed together, and very freely movable on one another. The anterior segment is known as the prothorax, the middle segment the mesothorax, and the posterior segment the metathorax.

In connection with the mesothorax and metathorax certain chitinous lateral plates are recognised. The exact homology of these plates is disputed, and the names applied to them differ so greatly that it would serve no useful purpose to describe them here. It will suffice to mention a large lateral plate (Plate X, ♀) attached to the metathorax, known by some as the *squama aliforme* (Tiraboschi), and by others as the *metathoracic epimeron* (Rothschild). This plate passes backwards over the abdomen and may readily be mistaken for a portion of an abdominal segment.

Numerous bristles, arranged in one or two vertical rows, are found on each segment of the thorax. In some species, *Ceratophyllus fasciatus* for example, a row of large teeth, similar to those surrounding the mouth parts in *P. felis*, is found along the posterior border of the prothorax (Plate XII). We shall call this row of large bristles the *prothoracic comb of bristles*. In some other species a similar comb is found on other segments of the thorax and even on the abdomen.

To each segment of the thorax a pair of legs is attached. Each leg is made up of the following parts; (1) a coxa, (2) a trochanter, (3) a femur, (4) a tibia, (5) a tarsus, the latter consisting of five segments (Plate IX, ♂). To the distal extremity of the last segment of each tarsus two claws are attached. The arrangement of the bristles on the various segments of the legs is of specific importance. In distinguishing the species much stress has been laid on the relative lengths of the various segments of the tarsus in the fore, mid, and hind legs.

*Abdomen.* The abdomen is made up of 9 segments (Plate XI), each segment consisting of a dorsal portion or tergum (tergite of Rothschild) and a ventral portion or sternum (sternite of Rothschild).

One or two vertical rows of bristles are present on each of the anterior tergites, while only a single row is found on some of the sternites.

If the dorsal margin of the abdomen is followed backwards, the observer will come to one or more large bristles which arise immediately anterior to and overhang a peculiar oval or heart-shaped organ. This organ is dotted over with small circular markings, from the centre of each of which a fine long hair projects, while surrounding each circle a number of more minute hairs will be found. This organ has been called

the pygidium and the large bristles, which overhang it, "the anti-pygidial bristles" (Plate X). The number and relative length of these bristles are of great utility in distinguishing the various species of fleas (Plate XII). Immediately posterior to the pygidium the genital organs and their addenda can be made out. These organs of course differ in the male and female.

#### *Determination of Sex.*

Male fleas are readily distinguished from female fleas as follows, (Plate X):—

(a) The male flea is usually smaller than the female; (b) the shape of the abdomen differs; and (c) within the abdomen of the male there are curved chitinous plates connected with the sexual organs which are wanting in the female.

As regards (b) the length of the dorsal margin of the abdomen in the male is considerably shorter than the ventral margin, so that the abdomen is tail tilted, as it were, while in the female the dorsal edge of the abdomen is quite as long as the ventral edge. Within the abdomen of the female, in place of the coiled chitinous plates found in the male, oval-shaped eggs can often be made out.

#### *Determination of Species.*

The exact species to which a particular flea belongs is usually determined by a careful examination of the generative organs and their appendages, but for practical purposes, when living fleas have to be identified, this method is impossible, in that it involves a careful dissection of the various parts which are more or less intimately associated with and lie concealed within the posterior abdominal segments.

As has already been remarked the abdomen consists of 9 segments. Considerable difference of opinion exists among entomologists as to the homology of these various segments, especially regarding the posterior segments. We have found it necessary to investigate this matter in connection with the description of *P. cheopis*.

It has generally been held that the first abdominal segment is represented only by the tergite and that the sternite of this segment is wanting, being fused with the *squama aliforme*, a portion of the meta-thoracic segment<sup>1</sup>. As will be seen from the account given below, we

<sup>1</sup> C. Tiraboschi, "Les rats, les souris et leur parasites cutanés," *Arch. de Parasitologie*, VIII. p. 220, 1904.



are not able to agree with this assumption. We consider that the first abdominal sternite is the one which has up to the present been recognised as the second by those who believe that the first sternite has been fused to the metathorax. Our enumeration of the abdominal segments is shown in the accompanying diagrams (Plate XI, left half). It is, however, necessary to detail more carefully the arrangements in the three posterior segments, viz. the 7th, 8th and 9th.

These segments are profoundly modified for sexual purposes and differ in the male and female.

The 7th abdominal tergite in both sexes bears the antipygidial bristles (Plate XI).

The 8th tergite differs markedly in the two sexes (Plate XI). In the male this tergite is very small, being only large enough to carry the stigmata or tracheal openings of the 8th abdominal segment, while in the female it is very greatly developed and covers over, not only a portion of the 9th tergite, but also the whole of the 7th, 8th and 9th sternites and the anal and vaginal orifices<sup>1</sup>. The arrangement of the bristles on this tergite in the female is of importance in classification.

The 9th tergite in both sexes carries the pygidium and an anal flap (Plate XI). On this flap in the female a prominent bristle can readily be made out, arising from an elevated pyramidal tubercle. We shall call this bristle the *pyramidal bristle*. The relative length of this bristle compared with the length of the antipygidial bristle is of use in distinguishing the species of fleas (Plates X and XI).

Passing now to the corresponding posterior sternites we see that the seventh abdominal sternite differs in the male and female. In the male this sternite (previously generally recognised as the 8th sternite) is largely developed. It bears on its surface a number of large bristles, the arrangement of these bristles varying in different species. This sternite conceals more or less completely the 8th and 9th sternites and the end of the penis (Plates X and XI)<sup>2</sup>. In the female the 7th sternite is a small, delicate, chitinous plate which forms the ventral wall of the external genital aperture. This plate contains also the opening of the duct to the spermatheca. Considerable care has to be taken to demonstrate this delicate sternite which, as far as we can ascertain from the literature available, has not been recognised by others who have worked at the subject. The position which it occupies relatively to the adjacent tergite and sternites can be understood from an examina-

<sup>1</sup> The parts have been separated out in the figures.

<sup>2</sup> The penis has not been drawn in the plates.



tion of Plate XI which gives both a lateral (I) and a posterior (II) view of the abdominal segments of a female. No bristles are found on this sternite.

The 8th abdominal sternite in the male and female is split mesially into two portions (Plate XI, right hand top figure).

In the male the 8th sternite corresponds to Rothschild's "boomerang-shaped organ," and also generally called by him the 9th sternite. The position of this sternite can readily be made out from Plates X and XI. Rothschild has drawn attention to it, as being of importance in distinguishing fleas.

In the female the 8th sternite, as is the case in the male, is split into two symmetrical portions, the two delicate flaps of chitin closing in postero-ventrally, more or less completely, the entrance to the oviduct (Plate XI, II). These flaps can be moved outwards and backwards during ovulation. The 9th sternite in the male has been called "the clasper." It is divided into two symmetrical portions completely separated from one another (Plate XI, top figure to the right). Certain portions of the clasper have received distinguishing names from Wagner and Rothschild, such as "the movable finger," "the immovable finger," and the "manubrium."

The 9th sternite in the female lies immediately below the anal flap of the 9th tergite and forms the lower boundary of the anal orifice. It is bilaterally symmetrical but is not split into two portions like the homologous sternite in the male (Plates X and XI).

#### DESCRIPTION OF *P. cheopis*.

We shall pass on now to a more detailed description of *P. cheopis*.

*Head.* Attention has already been drawn to the *maxillary palps* in the paper on the anatomy of the mouth parts (Plate X). As some stress has been laid on the measurements of the various segments of the palps in the differentiation of species, we have measured these segments in the case of twelve fleas of this species. The average measurements and the extreme variations in the length of each of the four segments are given in the following table. The measurements are given in  $\mu$  (microns).

	Average	Maximum	Minimum
1st or basal segment	64.7	74	56
2nd segment	71.7	86	62
3rd segment	51.4	67	46
4th or distal segment	91.9	120	78

A well-marked *eye* is present, situated on the anterior and inferior margin of the antennary groove (Plate X).

The shape of the *antennary groove* is different in the male and female. In the former it arises very near the summit of the head and is very much larger and longer than in the female (Plate X). It thus comes that the eye in the male lies somewhat lower in relation to the antennary groove, than in the female. Along the upper and posterior margin of the antennary groove, in the male only, a row of 13 small bristles will be found. Immediately posterior to the upper end of the antennary groove in the male a second groove (dorsal groove Plate X) will be noted, running along the whole of the narrow dorsal surface of the head as far back as its junction with the thorax. During copulation the male flea lies beneath the female and it is in this groove that the ventral surface of the anterior segments of the abdomen of the female lie during the act.

*Bristles on the head.* In both sexes the following arrangement of bristles on the head is noteworthy (Plate X). (1) One bristle (oral bristle) is situated on the antero-lateral surface of the head immediately above and external to the maxillary palp. (2) One bristle (ocular bristle) arises slightly anterior to and almost on a level with the upper margin of the eye. (3) Immediately behind the antennary groove a series of three bristles form a line running parallel with the groove and meeting another series of bristles which forms a vertical line passing upwards along the posterior margin of the head. These lines of bristles meet at an angle in a bristle which is common to both rows.

*Thorax.* On the thorax three vertical rows of bristles are found, one row along the posterior border of the tergite of each segment (Plate X). A double row of bristles is to be noted on the *squama alijorme* (Plate X, ♀). These bristles, since they appear to lie over the abdomen, should be distinguished from those found on the abdominal segments.

*Legs.* The arrangement of the bristles on the legs is characteristic.

*On the fore leg* (Plate X): on the external surface of the coxa a large number of bristles is found. There are no bristles on the internal surface of this segment. A number of small bristles are found on the outer surface of the femur, together with some larger bristles, both in front and behind, at the lower end of the segment. The tibia is furnished with a number of large stout bristles.

*On the middle leg* (Plate X) a few bristles occur along the anterior margin of the coxa. A few bristles are also found on the trochanter. A single row of about six bristles is found on the outer

surface of the femur, in addition to a row of bristles along the posterior border. This latter row terminates at the distal extremity in two curved bristles, one of which is considerably larger than the other. The tibia is furnished with a large number of stout bristles.

*On the hind leg* (Plate X) the outer surface of the coxa is covered with a number of bristles, especially its anterior portion. On the internal surface of this segment a curious row of small bristles, four to six in number, forms a line passing obliquely across the anterior portion of the segment (not shown in the Plate). A number of bristles are found on the trochanter. Along the posterior border of the femur a double row of small bristles is arranged, while on the external surface a row of six bristles is found and on the anterior edge two bristles are noteworthy. The tibia, as on the other legs, is furnished with a number of very large stout bristles.

The arrangement of the bristles in the five tarsal segments of all the legs can be recognised from the drawings.

A number of measurements have been made of the lengths of the femur, tibia and tarsal segments of 12 specimens. The maximum, minimum, and average length of each segment is recorded in the following tables. In giving these measurements it is well to remark that they were made from specimens which had been dehydrated and cleared.

The measurements do not represent the length of the longest diameter of each segment, but the length between the proximal and distal joints of each segment. The measurements are given in microns.

*Fore leg.*

Segments	Average	Maximum	Minimum
Femur	233.5	260	220
Tibia	161.8	180	130
1st or proximal tarsal segment	45.3	56	38
2nd tarsal segment	50.9	56	46
3rd tarsal segment	39.4	40	30
4th tarsal segment	29.6	35	28
5th or distal segment	86.9	93	80

*Mid leg.*

Segments	Average	Maximum	Minimum
Femur	277.2	306	240
Tibia	248.5	280	220
1st or proximal tarsal segment	78.4	93	64
2nd tarsal segment	109.7	118	80
3rd tarsal segment	61.7	80	52
4th tarsal segment	33.5	36	32
5th tarsal segment	94.2	106	80

*Hind leg.*

Segments	Average	Maximum	Minimum
Femur	375·8	400	340
Tibia	349·8	380	320
1st tarsal segment	239	260	220
2nd tarsal segment	168·3	184	146
3rd tarsal segment	91·4	100	86
4th tarsal segment	52	65	44
5th tarsal segment	111·7	120	100
Claw	73·7	96	46

*Abdomen.*

On the lateral surface of the tergite of each segment vertical rows of bristles will be made out. In the case of the first segment there are two such rows of bristles, while, in the case of segments 2 to 7 inclusive, a single row only is found, each row being composed of eight bristles. On the sternites of the 2nd to the 6th segments the number of bristles differs in the male and female. In the male three bristles are found, in the female four in each row. The number of these bristles, however, is not constant, five or six bristles on some of the segments of the female having been seen.

Near the posterior border of the 7th abdominal tergite a single pair of very long antipygidial bristles can be made out, i.e., one on each side.

The pyramidal bristle of the 9th tergite in the female is shorter than the antipygidial bristle (Plate X, ♀).

Of the remaining abdominal segments, the arrangement of the bristles on the 7th sternite in the male and the 8th tergite in the female is of importance.

On the 7th sternite of the male, in addition to a number of bristles scattered over its surface, two prominent bristles call for special notice. These bristles arise, one above the other, close to the posterior margin of the sternite and project almost vertically upwards. They are of course paired on the other half of the sternite (Plate X, ♂).

The 8th tergite in the female is covered with a large number of fairly large bristles: the number of these bristles varies slightly in different specimens, but the following rough arrangement can be made out (Plate X, ♀). Commencing from the lower end of the stigma, a series of bristles, increasing in size from above downwards, are



observed. These bristles, numbering from four to five, form a line which passes vertically downwards. This line of bristles meets another row at a very obtuse angle. From four to six bristles are found in this latter row, which passes in an oblique direction downwards and slightly backwards to nearly the lower and anterior border of the tergite. Here it joins another prominent row, of from 10 to 14 bristles, which passes in a curved direction upwards and backwards along the inferior and posterior border of the tergite. In the space enclosed between these rows of bristles three or four other bristles are found. Towards the upper extremity of the curved ascending row of bristles and close to the posterior edge of the tergite a row of from six to eight small stout bristles is found. These bristles run along one side of the external opening of the oviduct.

The shape and arrangement of bristles on the 8th sternite in the male are best understood from an examination of Plate XI (right half). It is again necessary to point out that this sternite is the one which Rothschild calls the 9th sternite or the "boomerang organ." It is split into two entirely separate halves. The shape of each half is like the letter L, the vertical portion having a number of small bristles along its posterior margin and a few at its summit.

The 8th sternite in the female, as in the male, is split into two halves. These plates, which are devoid of bristles, are composed of very thin, almost colourless, chitin, and cover in the postero-inferior portion of the external genital aperture (Plate XI, I and II).

The 9th sternite or clasper of the male is, as usual, characteristic of the species. The shape of the various parts of this sternite is best made out from Plate XI (right half).

The 9th sternite in the female is a small flap, covered with numerous bristles (not shown in the figure), and forms the inferior boundary of the anal orifice (Plate XI, I and II).

#### *Differentiation of P. cheopis from a few other species.*

It has of course been impossible to examine every flea used in our experiments in such detail as has been given above. We have found it necessary, however, to go into this detail because several species of flea have been described which apparently very closely resemble the one described above. We would instance in this connection, *P. pallidus* (Tasch.), *P. witherbyi* (Roths.), *P. nubius* (Roths.),



*P. cleopatrae* (Roths.), *P. chephrensis* (Roths.), *P. gerbilli* (Wag.), *P. murinus* (Tiraboschi), *P. philippinensis* (Schultze), etc., etc. We have examined a considerable number of the fleas (*P. cheopis*) used in our experiments in great detail, and have found them always conforming to the above description.

A number of specimens have also been sent to Rothschild and they have been kindly identified by him as *P. cheopis*. For practical purposes, therefore, and in view of the fact that we have never met any species resembling, but distinct from, *P. cheopis* on the rats in Bombay or in the Punjab, we have adopted the following more simple method of distinguishing *P. cheopis* from certain other species we have met with in connection with our work. These other species of flea are:—

(1) *Pulex irritans*, the human flea.

(2) *Ceratophyllus fasciatus*, the flea commonly found on rats in Europe.

(3) *Pulex felis*, the cat and dog flea.

(4) *Ctenopsylla musculi*, commonly found on mice and rats in various parts of the world.

(5) *Sarcopsylla gallinacea*, a flea commonly found on birds.

All the above-mentioned fleas (Plate XII) have at one time or another been met with on rats. They all have well-developed eyes, except *Ctenopsylla musculi*. Of the remainder, *Ceratophyllus fasciatus* and *P. felis* have a prothoracic comb of bristles, and the latter a perioral comb. These bristles are absent in *P. cheopis*, *P. irritans*, and in *Sarcopsylla gallinacea*, but the latter flea is readily distinguished from the other two by its angular-shaped head and its largely developed mandibles.

We are thus left with *P. cheopis* and *P. irritans*, which can be differentiated from one another as follows:—

1. *P. cheopis* is small and light-coloured when compared with *P. irritans*.

2. The number of bristles on the head is greater in *P. cheopis* than in *P. irritans*, i.e. there is only one bristle on the posterior portion of the head in *P. irritans*, namely, the one which is found at the posterior and inferior angle of the head and corresponds in situation to the bristle which is common to the two rows of bristles found in this posterior portion of the head of *P. cheopis*. Moreover, the ocular bristle in *P. cheopis* is situated nearly on a level with the upper border of the eye, while in *P. irritans* it arises nearer to the lower margin of the eye.

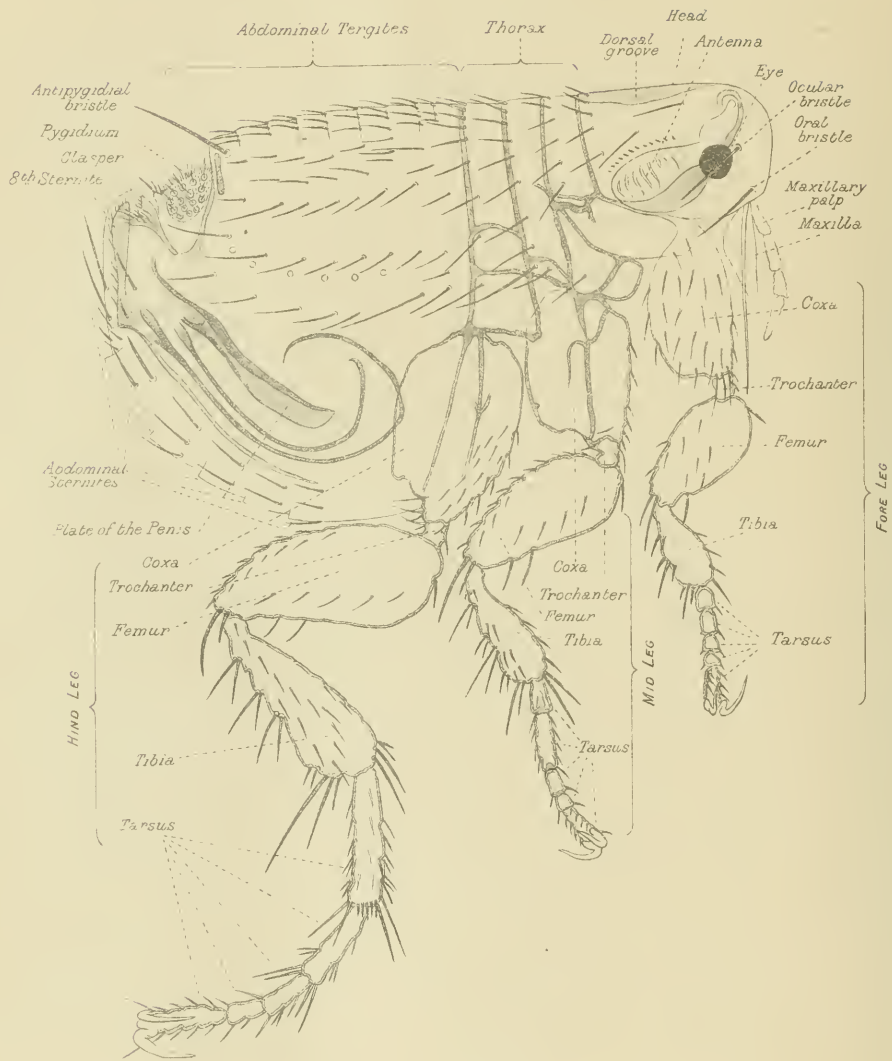
3. The antipygidial bristles in *P. cheopis* are longer than those found in *P. irritans*.

4. In the female *P. cheopis* the antipygidial bristle is longer than the pyramidal bristle of the 9th tergite, while in *P. irritans* the pyramidal bristle of the 9th tergite is as long as, if not longer than, the antipygidial bristle.

5. In the case of males the shape of the clasper at once distinguishes the fleas from one another.

6. The shape and size of the claws are different in the two species. They are small in *P. cheopis* and large in *P. irritans*.

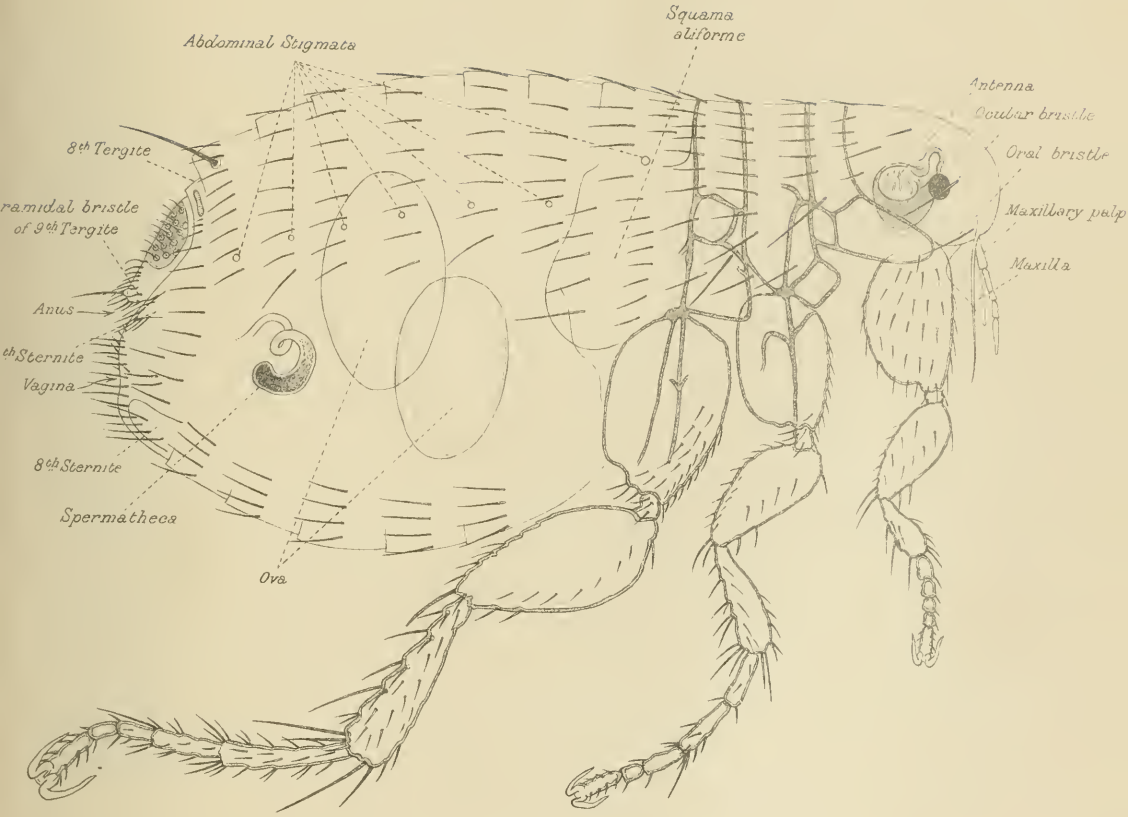
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♂ PULEX CHEOPIS, ROTHSCHILD.

R. A. Turkhad M.B. Edin.

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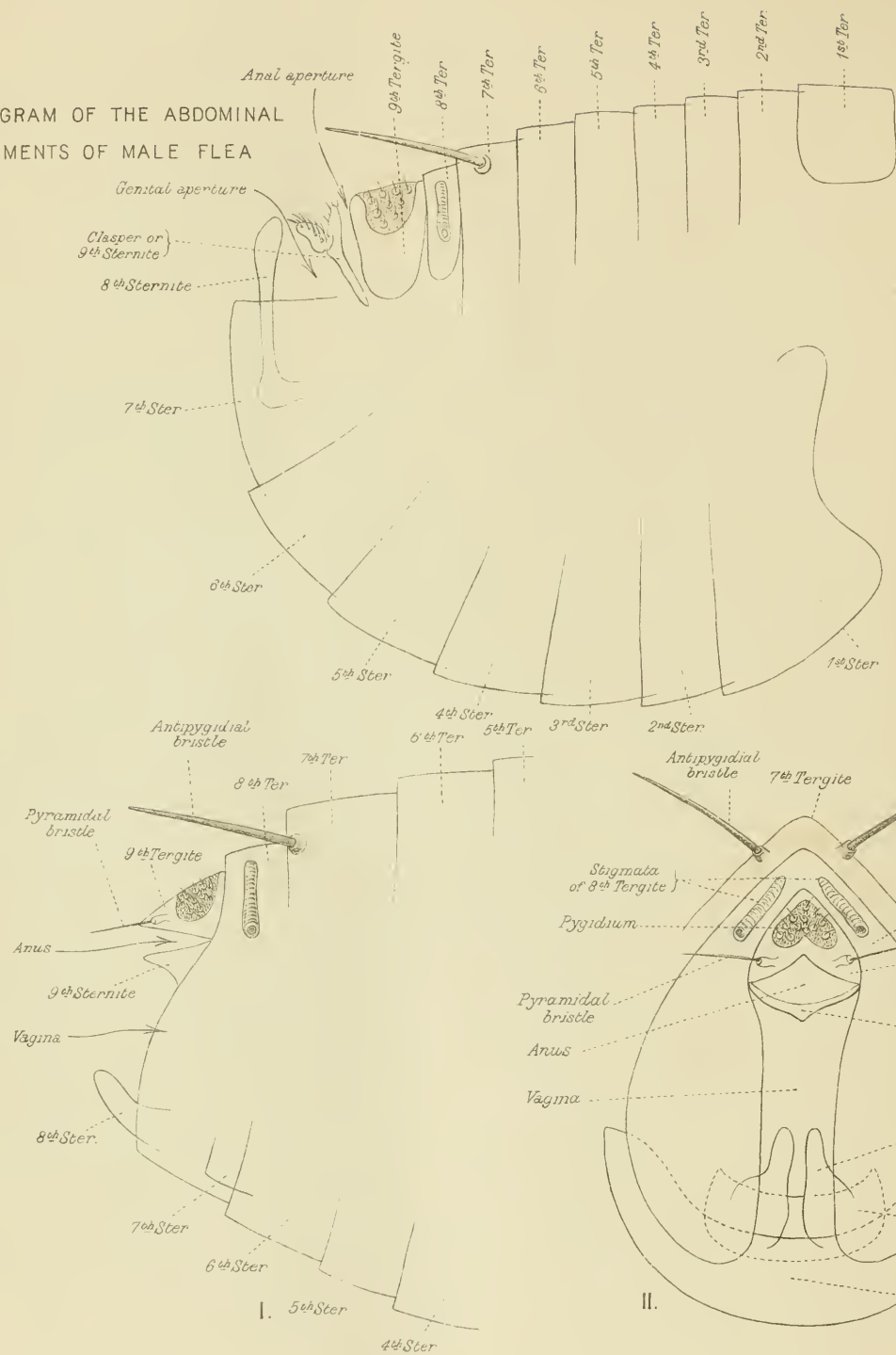
♀ PULEX CHEOPIS, ROTHSCHILD







GRAM OF THE ABDOMINAL  
MENTS OF MALE FLEA

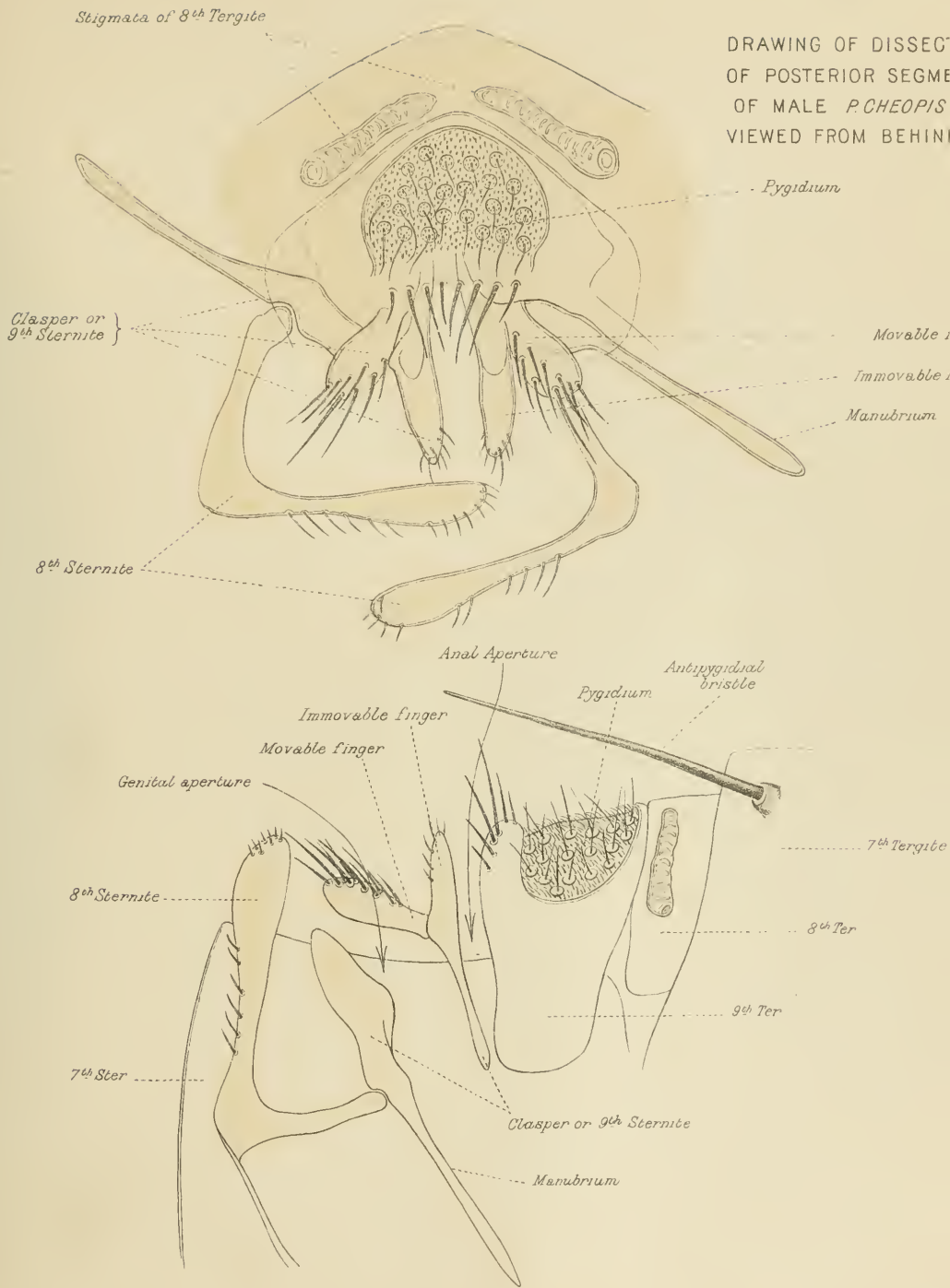


D A Turkhud, M B Edin

DIAGRAM OF THE POSTERIOR ABDOMINAL SEGMENTS OF A FEMALE FLEA

I. VIEWED FROM THE SIDE

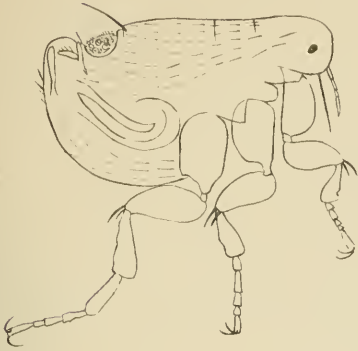
II. VIEWED FROM BEHIND



DIAGRAMATIC REPRESENTATION OF POSTERIOR ABDOMINAL SEGMENTS OF MALE *P. CHEOPIS*, VIEWED FROM THE SIDE





♂ *Pulex cheopis*.

♂ *Pulex irritans*.



♂ Pulex felis



♂ *Ceratophyllus fasciatus*



♂ *Ctenopsylla musculi*



♂ *Sarcopsylla gallinacea*.





## XIX. ON THE NATURAL OCCURRENCE OF CHRONIC PLAGUE IN RATS.

### I. INTRODUCTION.

In a previous paper (vol. VI. p. 530) an account was given of seven rats presenting the appearances of chronic plague which were obtained in the Punjab villages, Kasel and Dhand, during the months immediately preceding the epizootic. Since the year devoted to the study of human and rat plague in these villages has now been completed, we are in a position to put on record further observations of chronic plague in rats and to discuss the possible bearing of these observations upon the question of the seasonal recurrence of the infection in an acute form. It will be recalled that the lesions in the seven rats already described were in every instance situated within the abdomen. Further examples of rats with similar lesions are included in the present paper, but in addition reference will be made to certain rats in which the lesion was peripheral, i.e. in the sub-maxillary, axillary or inguinal regions. We shall refer in future to the former type as "visceral" and to the latter as "peripheral": abscesses in the pelvic lymphatic glands are, for reasons given subsequently (p. 466), to be classed as essentially peripheral. We have in all met with 45 cases, of which 17 were visceral and 28 peripheral. The lesions in every instance consisted of circumscribed caseous nodules or abscesses, and with eight exceptions constituted the only pathological change found. All the rats thus affected were *Mus rattus*, which constitutes practically the entire rat population of the villages examined.

The condition seems to be clearly different from that described by Hunter (*Epidemic and Epizootic Plague*, 1904, pp. 77 and 84), who in Hongkong found a large number of rats suffering from chronic plague. These animals were much emaciated and suffered from chronic diarrhoea: on section necrosed areas of cheesy material were found in the lymphatic glands and viscera, containing few plague bacilli but capable of giving

TABLE I.

*Chronic plague rats obtained from Kasel village during the year 29. xi. 05—14. xii. 06.**Chronic plague rats obtained before the first acute plague rat was taken, i.e. on 2. iv. 06.*

Serial No.	No. of rat	Date of examination	Where caught	Weight in grs.	Sex	Situation of principal lesion	Microscopical appearances	Cultural tests	Animal tests
1	403	9. xii. 05	House No. 138	—	—	Abscess in spleen	Numerous bacilli like <i>B. pestis</i>	Like <i>B. pestis</i> on agar	Guinea-pig died in 8 days of plague.
2	754	12. xii. 05	376	—	—	Abscess in spleen	A few plague-like bacilli	All positive *	Guinea-pig recovered after local reaction and buboes.

*Rats obtained in the period dating from the first to the last acute plague rat, i.e. from 2. iv. 06 to 17. vii. 06.*

Note :—Trapping stopped between 24. xii. 05 and 20. ii. 06.

3	4661	6. iv. 06	119	130	F preg.	Necrotic node in spleen	Many plague-like bacilli	All positive	Guinea-pig died in 4 days of plague.
4	4739	17. iv. 06	82	155	M	Left axillary abscess	Many plague-like bacilli	—	Guinea-pig chloroformed on 13th day : plague.
5	4765	18. iv. 06	118	140	M	Left submaxillary abscess	Fairly numerous plague-like bacilli	Like <i>B. pestis</i> on agar	None made.
6	5470	21. v. 06	31	130	F	Left axillary and right submaxillary abscesses	Submaxillary—abundant plague-like bacilli. Axillary—several clumps plague-like bacilli	—	None made.
7	5485	23. v. 06	109	110	F preg.	Left submaxillary bubo	Abundant plague-like bacilli and involution forms	Like <i>B. pestis</i> on agar	None made.
8	5489	23. v. 06	105	165	M	Right and left axillary abscesses	Left axillary—numerous plague-like bacilli. Right axillary—a few plague-like bacilli	Like <i>B. pestis</i> on agar	None made.
9	5567	30. v. 06	439	155	M	Right submaxillary bubo	A few plague-like bacilli	Like <i>B. pestis</i> on agar	Guinea-pig died in 7 days of plague.
10	5597	31. v. 06	490	95	F	Right inguinal and right pelvic buboes	Inguinal—many plague-like bacilli	(From pelvic bubo). Like <i>B. pestis</i> on agar	None made.

11	5725	11. vi. 06	1206	55	F	Right axillary bubo, 2 abscesses in spleen	Bubo: many involution forms like <i>B. pestis</i>	From bubo—like <i>B. pestis</i> on agar	Guinea-pig died in 5 days of plague.
12	5774	15. vi. 06	138	135	F preg.	Right submaxillary bubo	A few plague-like bacilli	Like <i>B. pestis</i> on agar	Guinea-pig died in 5 days of plague.
13	5781	16. vi. 06	272	125	F	Left axillary abscess	No bacilli seen	Contaminated	Guinea-pig chloroformed in 7 days: plague.
14	5827	21. vi. 06	552	140	M	Left submaxillary abscess	Fairly numerous bacilli-like involution forms of <i>B. pestis</i>	Like <i>B. pestis</i> on agar	Guinea-pig died in 3 days of plague.
15	5831	21. vi. 06	508	40	M	Right inguinal abscess	Many bacilli, none like typical <i>B. pestis</i>	Contaminated	Guinea-pig died in 5 days of plague.
16	5924	29. vi. 06	715	45	M	Necrotic nodule in spleen, abscess in abdominal wall	Abscess: some plague-like bacilli	Like <i>B. pestis</i> on agar	Guinea-pig died in 3 days of plague.
17	6159	13. vii. 06	208	140	F preg.	Mesenteric abscess	No bacilli seen	All positive	Guinea-pig died in 5 days of plague.
<i>Rats obtained in the period dating from the last acute plague rat to the end of the year's investigation on 14. xii. 06.</i>									
18	6927	23. viii. 06	525	145	M	Necrotic nodule in spleen	No bacilli seen	Sterile on agar	Guinea-pig chloroformed in 4 days, cultures proved to be plague*.
19	7097	6. ix. 06	1063	130	F preg.	Left inguinal and left pelvic abscesses	Inguinal — no bacilli seen. Pelvic—very numerous like <i>B. pestis</i> & some involution forms	Pelvic—all positive	Guinea-pig died in 6 days of plague.
	7667	6. x. 06	899	135	F preg.	Abscess in spleen	No bacilli seen	Like <i>B. pestis</i> on agar	Guinea-pig died in 11 days of plague, cultures proved to be plague.
	8279	28. x. 06	138	150	M	Abscess in spleen	A few bacilli unlike plague	All positive	Guinea-pig died in 3 days of plague.
	8580	5. xi. 06	422	145	F	Left pelvic abscess	A few clumps of plague-like bacilli and involution forms	All positive	Guinea-pig died in 3 days of plague.
	8834	13. xi. 06	673	140	F	Abscess in spleen	Very few plague-like bacilli	All positive	Guinea-pig died in 3 days of plague.
	9713	5. xii. 06	564 or 576	190	M	3 mesenteric abscesses	A few bacilli but not typical of plague	All positive	Guinea-pig died in 3 days of plague.

Note:—The first human case was attacked on 9. iii. 06 and the last on 6. vii. 06.

In the animal tests in this Table and in Tables II and III the guinea-pigs were inoculated by the cutaneous method.

\* I.e. (1) growth-characteristics on agar, (2) staphylococcus test, (3) pathogenicity to guinea-pig with characteristic post-mortem appearances.



TABLE II.

*Chronic plague rats obtained from Dhand village during the year 4. xii. 05—3. xii. 06.**Chronic plague rats obtained before the first acute plague rat was taken on 27. i. 06.*

Serial No.	No. of rat	Date of examination	Where caught	Weight in grs.	Sex	Situation of principal lesion	Microscopical appearances	Cultural tests	Animal tests
1	1255	18. xii. 05	House No. 110	110	F	Multiple abscesses in liver and mesentery	No bacilli seen	All positive	None made.
2	1235	18. xii. 05	378	407	—	Pelvic abscess	A few plague-like bacilli	" "	Guinea-pig died in 5 days of plague.
3	1779	24. xii. 05	623	105	M	Mesenteric abscess	No bacilli seen	" "	" " 6 "
4	2356	11. i. 06	192	—	—	Pelvic abscess	" "	" "	" " 9 "

*Rats obtained in the period dating from the first to the last acute plague rat, i.e. from 27. i. 06 to 21. iv. 06.*

5	3053	14. ii. 06	492	130	M	Pelvic bubo	Numerous small bacilli	All positive	Guinea-pig remained healthy.
6	3424	27. ii. 06	364	165	M	Submaxillary abscess	Many clumps of plague-like organisms	—	Guinea-pig died in 9 days of plague.
7	4022	12. iii. 06	321	110	M	Right axillary bubo	One group of plague-like bacilli	—	" " 9 "
8	4116	14. iii. 06	439	55	F	Necrotic nodule in spleen	No bacilli seen	—	" " 4 "
9	4639	6. iv. 06	186	35	F	Right submaxillary abscess	Many plague-like bacilli	Like <i>B. pestis</i> on agar	" " 5 "
10	4696	12. iv. 06	371	110	M	Right submaxillary bubo	No bacilli seen	—	" " 4 "

*Rats obtained in the period dating from the last acute rat to the end of the year's investigation on 3. xii. 06.**Note:—*Trapping stopped between 12. iv. 06 and 22. v. 06.

11	5695	8. vi. 06	262 A	155	M	Left submaxillary abscess	Several clumps of plague-like bacilli	Like <i>B. pestis</i> on agar	Guinea-pig died in 4 days of plague.
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*Note:—*Trapping stopped between 25. vi. 06 and 14. ix. 06.

The first human case was attacked on 6. ii. 06 and the last on 2. v. 06.

TABLE III.

Chronic plague rats obtained from Mianpur, Basirki and Dhaul.

I. Rats obtained in Mianpur.						
Serial No.	No. of rat	Date of examination	Weight in grs.	Sex	Situation of principal lesion	Microscopical appearances
1	5872	27. vi. 06	70	F	Left submaxillary abscess	Numerous plague-like bacilli and some involution forms
2	5916	29. vi. 06	55	M	Submaxillary abscess	No bacilli seen
3	5936	30. vi. 06	115	F	Right axillary abscess	" "
4	5937	30. vi. 06	130	M	Right submaxillary abscess	Fairly numerous plague-like bacilli with involution forms
5	5979	3. vii. 06	165	M	Abscess in spleen	Fairly numerous plague-like bacilli
6	6014	4. vii. 06	110	M	Right submaxillary abscess	Some plague-like bacilli
7	6171	13. vii. 06	120	F	Right axillary abscess	No bacilli seen
Cultural tests						
All positive						
Animal tests						
Guinea-pig died in 6 days of plague.						
" " 4 " "						
Guinea-pig chloroformed in 8 days.						
Guinea-pig died in 3 days of plague.						
" " 6 " "						
Like <i>B. pestis</i> on agar						
" " 5 " "						
Like <i>B. pestis</i> on agar						
Sterile on agar						
" " 2 " "						
Culture from second guinea-pig proved to be plague.						
Guinea-pig chloroformed in 10 days. Culture inoculated into rat. Culture from rat proved to be plague.						
Guinea-pig died in 3 days of plague.						
Cultural tests						
All positive						
Animal tests						
Rat died in 2 days after subcutaneous inoculation of culture from abscess.						

II. Rats obtained in Basirki.

No bacilli seen

All positive

Guinea-pig chloroformed in 10 days. Culture inoculated into rat. Culture from rat proved to be plague.

Guinea-pig died in 3 days of plague.

III. Rat obtained from Dhaul.

No bacilli seen

All positive

Rat died in 2 days after subcutaneous inoculation of culture from abscess.

Note :—(1) In Mianpur trapping was commenced on 25. vi. 06 and stopped on 12. ix. 06. 309 rats were trapped during this period. No acute plague rats were obtained.

The village was again trapped on 4. xii. 06 and 5. xii. 06, 10 rats were secured.

The first human plague death occurred on 7. v. 06 and the last on 23. v. 06.

(2) Basirki was trapped on 20. x. 06 and 21. x. 06, 107 rats were obtained.

The village was retrapped from 14. xi. 06—17. xi. 06, 153 rats were obtained.

No human case was reported in this village during the plague season 1905—06.

(3) Dhaul village was not trapped.

The first human plague death occurred on 22. xi. 05 and the last on 20. ii. 06.

rise to acute plague when administered to healthy rats. He found that such animals were caught more frequently in the interval between than during the epizootics of acute plague. Our rats on the other hand showed no signs of general illness, and in only one instance was emaciation noticed.

The details of all the cases are given in Tables I, II and III in which the rats are arranged according to the village in which they were captured and in order of the date of capture.

## II. THE POST-MORTEM APPEARANCES OF THE RATS<sup>1</sup>.

### A. *Chronic Plague of the visceral type (17 cases).*

#### 1. *Splenic Nodules and Abscesses (12 cases).*

Out of 22 rats with lesions in the abdomen the spleen was the seat of the lesion in twelve.

*No. 3, Table I.* The posterior half of the spleen contains a yellowish hard nodule about the size of a pea; the nodule is adherent to the abdominal wall.

*No. 18, Table I.* The spleen contains a small cheesy nodule about 2 mm. in diameter at the junction of the middle and anterior third. The spleen is attached to the abdominal wall by a fibrous band.

*No. 8, Table II.* The anterior end of the spleen contains a necrotic nodule about the size of a small pea adherent to the great omentum: there is a deposit of lymph on the posterior third of the external surface. The liver shows numerous white points.

*No. 16, Table I.* There is a grayish nodule in the posterior third of the spleen. In the abdominal wall opposite to the nodule and attached to it by a fibrous band there is a very small thick-walled abscess. The rat shows some emaciation.

*No. 2, Table I.* A small abscess in the anterior end of the spleen.

*No. 1, Table I.* An abscess in the spleen containing cheesy pus; localised thickening of the capsule.

*No. 20, Table I.* In the anterior third of the spleen there is an abscess about 6 mm. in diameter, projecting from the internal surface and containing cheesy pus.

*No. 21, Table I.* The anterior third of the spleen contains an

<sup>1</sup> With the exception of the lesions described the rats appeared to be healthy.

abscess 8 or 9 mm. in diameter, with moderately thin walls; it projects from the internal surface of the spleen and contains cheesy pus.

*No. 23, Table I.* An abscess about 20 mm. in diameter is situated in the anterior third of the spleen projecting from the external surface. It has fairly thick walls. The spleen is granular and the kidneys have a pale and mottled appearance.

*No. 5, Table III.* The spleen contains a large bulging abscess at the junction of the posterior and middle third. The abscess has a fairly thick fibrous wall and is partially covered by a thin layer of spleen tissue. A thin fibrous band extends from the spleen in the neighbourhood of the abscess to the abdominal wall; the abscess is connected also by a similar band to the upper and outer surface of the left kidney, the capsule of which has a local thickening. The spleen otherwise appears normal.

*No. 11, Table I.* The posterior end of the spleen contains a small abscess adherent to the stomach. In the anterior third of the spleen there is another small abscess adherent by means of a band of fibrous tissue to the posterior wall of the abdomen. There is a right axillary bubo. The liver shows several white necrotic spots.

*No. 9, Table III.* This rat shows three abscesses, all containing thick pus:—

(1) One the size of a small walnut in the posterior half of the spleen projecting from the internal surface, and adherent to the left kidney: (2) an abscess the size of a large pea in the anterior third of the spleen projecting forwards: (3) an abscess in the liver similar in size to the last.

## 2. *Mesenteric Abscesses (5 cases).*

*No. 3, Table II.* There is an abscess in the mesentery adherent to the spleen.

*No. 10, Table III.* A small abscess containing cheesy pus is situated in the mesentery and is attached to the upper end of the spleen by a band of fibrous tissue.

*No. 17, Table I.* There is a thick-walled abscess about the size of a large pea in the mesentery near the rectum; the abscess contains fairly thick pus. The spleen has two thin fibrous adhesions each about 5 mm. long attaching it at two points to the abdominal wall.

*No. 24, Table I.* There are three abscesses in the mesentery. The largest, about the size of a small walnut, lies below the stomach and is adherent to the small intestine and to the coecum. The second is oval

## 464 *Natural Occurrence of Chronic Plague in Rats*

in shape, measuring about  $8 \times 4$  mm. and lies close to the coecum. A third abscess about the same size as the first is situated in the great omentum; it has no adhesions to any of the organs. All these abscesses contain thick cheesy pus.

*No. 1, Table II.* There are numerous abscesses in the liver and mesentery.

### *B. Chronic Plague of the peripheral type (28 cases).*

Out of 27 rats with peripheral abscesses the lesion was pelvic in 5, submaxillary in 13, axillary in 6, inguinal in 2, and in one there was an abscess in two situations. The details of the pelvic abscesses are as follows:—

*No. 2, Table II.* One of the retroperitoneal pelvic glands is full of cheesy pus.

*No. 4, Table II.* One of the pelvic glands is converted into an abscess.

*No. 5, Table II.* There is a pelvic bubo containing pus. The kidneys are pale and distended with urine; the bladder and ureters are also distended with urine, the result evidently of pressure by the abscess on the neck of the bladder.

*No. 19, Table I.* There is a very small left inguinal bubo containing a drop of pus and a left pelvic abscess about the size of a hazel-nut with cheesy purulent contents.

*No. 22, Table I.* The left pelvic gland is converted into an abscess about the size of a pea adherent to the posterior abdominal muscles and containing thick pus.

In 3 only of the remaining 22 rats was anything abnormal noted beyond the abscess:—

*No. 6, Table I.* Left axillary and right submaxillary purulent buboes; the spleen shows a few yellow nodules.

*No. 10, Table I.* Right inguinal and right pelvic necrotic buboes; the liver shows several small white necrotic nodules.

*No. 12, Table I.* Right submaxillary bubo; the spleen contains a few white nodules.

There remains for description *Rat No. 8, Table III.* This rat had an abscess containing thick cheesy pus in the middle of the sternum, connected with the bone. In close relation to it there were 2 subcutaneous abscesses with similar contents.



3. *Microscopical Appearances.*

Plague bacilli were not abundant in the lesions: in 17 cases none were found, while in 10 instances they were noted as being few, and were abundant in 18 only. Cultures were made in 36 instances and plague bacilli recovered in 32.

The heart-blood and normal tissue of the spleen in these animals showed no plague bacilli microscopically, and gave negative results on cultural examination. The heart-blood and spleen of some of them were inoculated into guinea-pigs without result, except in the case of No. 19, Table I. The following are the notes of this case:—There is a very small left inguinal bubo containing a drop of pus and a left pelvic bubo about the size of a hazel-nut with cheesy purulent contents. On microscopical examination, very numerous plague-like bacilli and some involution forms are seen in the pus from the pelvic bubo, while no bacilli can be seen in the pus of the inguinal bubo nor in the heart-blood nor spleen. A guinea-pig inoculated cutaneously with the pus from the pelvic abscess died in 5 days and a guinea-pig inoculated by the same method with the spleen died in 3 days. Judging from the post-mortem appearances we think it probable that this rat was passing from a subacute to the chronic stage of the disease.

4. *Virulence of the Bacilli.*

In the great majority of cases the bacilli in the lesions were virulent. The following table gives the result of the cutaneous inoculation of guinea-pigs with material from the lesions or with cultures from the pus. Of the 38 cases examined in this way, the test animals died of acute plague (5 days or less) in 21 (55 per cent.); the details are shown in the next table.

TABLE IV.

Guinea-pigs which remained healthy	...	1
„ „ were ill but recovered	...	1
„ „ died in 2 days	...	1
„ „ „ 3 „	...	8
„ „ „ 4 „	...	5
„ „ „ 5 „	...	7
„ „ „ 6 „	...	4
„ „ „ 7 „	...	1
„ „ „ 8 „	...	1
„ „ „ 9 „	...	3
„ „ died after 9 days	...	1
„ „ were killed and showed plague	...	5

## 466 *Natural Occurrence of Chronic Plague in Rats*

One rat inoculated with a culture from a mesenteric abscess died of acute plague in 2 days.

In eight cases rats were fed with the lesions, and in two instances died of acute plague. It is moreover to be noted that the material used contained few or no plague bacilli on microscopical examination.

TABLE V.

*Feeding experiments with the lesions of chronic plague rats.*

No.	Lesion	Microscopical examination	Result	Remarks
9713	Mesenteric abscess	1 or 2 bacilli not typical	Died in 4 days	Mesenteric bubo ; plague
9713	„ „	1 or 2 bacilli not typical	Chloroformed in 8 days	P.-M., nothing seen
8834	Spleen and splenic abscess	Very few plague-like bacilli	Died in 3 days	P.-M. plague, left sub-maxillary buboacute plague
7097	Very small inguinal bubo	No bacilli seen	Died in 9 days	Nothing P.-M.
7667	Spleen and splenic abscess	No bacilli seen	Chloroformed in 15 days	P.-M., nothing seen
8279	Spleen and splenic abscess	A few bacilli not like <i>B. pestis</i>	Chloroformed in 21 days	„ „ „
8890	Abscesses in spleen and liver	A few plague-like bacilli	Chloroformed in 20 days	„ „ „
5695	Submaxillary abscess	Several clumps of <i>B. pestis</i> -like bacilli	Chloroformed in 33 days	„ „ „
2356	Pelvic abscess	No bacilli seen	Killed in 14 days	„ „ „

### 5. *Origin of the Lesions in Chronic Plague Rats.*

(1) *Peripheral abscesses.* These were invariably in the situation of the peripheral lymphatic glands and undoubtedly originated from buboes. In 14 animals out of 22 (64 per cent.) the abscesses were in the submaxillary region. This corresponds with the distribution of the primary buboes of acute rat plague (see above, p. 382), and indicates that the infection takes place by the same route in the two classes; reasons have been already given which suggest that the route in question is by the skin through the agency of rat fleas.

(2) *The pelvic abscesses.* We think that these abscesses originate from an infection of the skin of the hind limbs or of the lower part of the body. The reasons for this conclusion are (1) that the lymphatics of this area drain into the pelvic glands, and (2) that we have repeatedly observed instances of implication of the pelvic glands following experimental inoculation of plague bacilli in the hind limbs of rats.

Moreover, not a few instances of pelvic buboes have been observed in rats dead of plague as the result of the transmission of the disease by infected rat fleas, i.e. by a skin infection. In this connection a rat cited above (vol. VI, p. 533) as an instance of chronic plague produced by experimental flea transmission is of interest. All the organs in this case were healthy but there was a bubo in the right pelvis containing virulent plague bacilli.

(3) *The splenic and mesenteric abscesses.* The origin of the splenic and mesenteric abscesses is obscure, and susceptible of several explanations.

The theory to which we incline is that the bacilli in the first instance find a lodgement in the spleen, either in the course of a septicaemia or as an embolus from, e.g., a bubo. They here give rise to a local necrosis which, owing to the prolonged life of the rat, is followed by a local inflammatory reaction. In this way an encapsuled abscess and a localised perisplenitis are produced. Subsequently there is an extension by direct contiguity to the mesentery, the connecting inflammatory links being later reduced to fibrous bands which may in their turn altogether disappear. The points in the morbid anatomy which especially favour this version are the facts that of 17 abscesses among the abdominal viscera, the spleen was involved in 12, and in 3 out of 5 cases of mesenteric abscesses the lesion was connected to the spleen by a definite adhesion.

It is, however, possible that these splenic and mesenteric abscesses are the few remaining representatives of multiple localised foci of plague bacilli, and that their distribution is an expression of individual peculiarities in infection and resistance by the different viscera. It is also possible that the mesenteric abscesses represent encapsuled remains of a generalised infection of the peritoneum, arising in the first instance from e.g. the spleen or bowel.

It might appear at first sight that the mesenteric abscesses originated from an intestinal infection resulting from feeding on plague-infected material. As against this view, however, the lesions were not the same as those in certain rats with chronic plague induced by experimental feeding on plague-infected material, and the appearances presented by the abscess did not support the view that they originated in lymphatic glands, though this origin cannot of course be altogether excluded. The buboes in the rats infected by feeding were always in the sites of known lymphatic glands, while the abscesses in the present

## 468 *Natural Occurrence of Chronic Plague in Rats*

series of animals occurred in places where no lymphatic glands could be demonstrated in normal animals.

### 6. *Chronic Rat Plague in Bombay.*

It is a remarkable circumstance that chronic plague rats, either with abdominal or peripheral abscesses, seem scarcely to exist in Bombay. Among more than one hundred thousand live and dead rats (*M. decumanus* and *M. rattus*) examined by us, only one has been found with lesions corresponding to those under discussion. The details of this rat are briefly as follows.

*Mus decumanus*: 27. VIII. 06—weight 310 grammes—trapped alive. The spleen contains a small abscess about the size of a large pea with semi-liquid purulent contents. The spleen is attached by a fibrous band to an abscess which is adherent to the abdominal wall above the left kidney and to the mesentery. A third abscess is situated between the spleen and the left kidney and is adherent to both these organs. A fourth abscess is situated below the left kidney and in close relation to the cecum. Microscopically the abscesses show a very large number of organisms, both coccal and bacillary forms. A guinea-pig inoculated cutaneously with the pus of one of the abscesses remained healthy, but cultures on Conradi-Drigalski medium of the pus from the splenic abscess furnished a pure growth of *B. pestis*.

We can put forward no adequate explanation of the reason for the relative frequency of chronic rat plague in the Punjab villages and for its rarity in Bombay.

### 7. *The Relation of Chronic Rat Plague to acute Epizootic Plague.*

The relation in time between the cases of chronic plague and the prevalence of acute epizootic plague is shown in the following table (Table VI) as far as the two villages chiefly investigated are concerned.

It seems fairly clear from these figures that the peripheral type occurs especially during the progress of the acute epizootic, and chiefly during its decline, while the visceral type is found more commonly in the intervals between the epizootics. Thus four-fifths of the peripheral type and only one-half of the visceral cases occurred during the prevalence of the epizootic.

We have little evidence which might bear on the aetiology of this interesting condition; and still less to explain the varying seasonal incidence of the two types. A diminution of the virulence

of the bacilli would appear to be excluded by the experiments with the organisms in the abscesses already detailed (p. 465). *If observations on the rats of Bombay may with propriety be transferred to Punjab rats,* an increase in the immunity of the rats may be excluded. There are reasons for thinking that the mode of infection of the peripheral group of cases is the same as that which operates in acute plague, and for these a diminution of the quantity of bacilli inoculated is a suggestion which receives support from the observed diminution in the

TABLE VI.

Date	Kasel			Dhand		
	Acute	Chronic peripheral	Chronic visceral	Acute	Chronic peripheral	Chronic visceral
Dec. 1905	—	—	2	—	1	2
Jan. 1—20, 1906	—	—	—	—	1	—
„ 21—27	—	—	—	1	—	—
„ 28—Feb. 3	—	—	—	3	—	—
Feb. 4—10	—	—	—	2	—	—
„ 11—17	—	—	—	—	1	—
„ 18—24	—	—	—	1	—	—
„ 25—Mar. 3	—	—	—	3	1	—
Mar. 4—10	—	—	—	3	—	—
„ 11—17	—	—	—	1	1	1
„ 18—24	—	—	—	3	—	—
„ 25—31	—	—	—	3	—	—
April 1—7	5	—	1	6	1	—
„ 8—14	5	—	—	—	1	—
„ 15—21	15	2	—	2	—	—
„ 22—28	22	—	—	—	—	—
„ 29—May 5	44	—	—	—	—	—
May 6—12	48	—	—	—	—	—
„ 13—19	42	—	—	—	—	—
„ 20—26	23	3	—	—	—	—
„ 27—June 2	12	2	—	—	—	—
June 3—9	5	—	—	—	1	—
„ 10—16	9	2	1	—	—	—
„ 17—23	2	2	—	—	—	—
„ 24—30	3	—	1	—	—	—
July 1—7	1	—	—	—	—	—
„ 8—14	1	—	1	—	—	—
„ 15—21	1	—	—	—	—	—
„ 22—31	—	—	—	—	—	—
August	—	—	1	—	—	—
September	—	1	—	—	—	—
October	—	—	2	—	—	—
November	—	1	1	—	—	—
Dec. 1—14	—	—	1	—	—	—



prevalence of fleas in the Punjab coincident with the decline of acute plague<sup>1</sup>. The remarkable difference between the prevalence of chronic plague in Bombay and in the Punjab villages cannot be explained. In Bombay the intervals between the acute epidemics are filled in with a small but continuous prevalence of acute plague in rats and men, while in the Punjab they are occupied by scattered instances of chronic rat plague without any human plague. As far as can be ascertained, the bacilli and the fleas in the two places show no suggestive difference in any respect.

We may next proceed to consider whether rats affected with chronic plague, such as we have described, possess any significance as a source of bacilli in the recrudescence of acute epizootic plague. The bacilli in these lesions seem strictly localised, and it would appear that they could only become available for other animals in one of two ways:—(1) the chronic lesion lights up into an acute condition, or (2) rats become infected with acute plague by eating animals with chronic plague.

Considerations based upon the morbid anatomy of the lesions in chronic plague appear to us to bear against the view that the condition may become acute, e.g. by an abscess rupturing into the peritoneum or into a vein. Adhesions seem to form readily so as to limit the lesion, and most of the abscesses had thick fibrous walls. There is too no direct evidence of any such "lighting up": no rats have been found with lesions suggestive of such an occurrence, which would, however, not be without parallels in human pathology.

We have no reason to think that rats contract acute plague in nature by feeding on the carcasses of chronic plague rats. Rats infected by feeding show mesenteric buboes (p. 373), and neither in Bombay nor the Punjab have these been found in any rat dead of acute plague.

It is therefore not easy to see how these chronic abdominal abscesses can afford a source of origin for an acute epizootic. The fact, however, remains that they are the only place where plague bacilli are known to occur in the intervals between the epidemics of acute rat and human plague in the Punjab, and direct experiment has demonstrated that the abscesses may give rise to acute plague when fed to healthy rats.

The evidence at present available although suggestive justifies no positive conclusion as to the epidemiological importance of these rats.

<sup>1</sup> This will be fully described in a later paper.

8. *Summary and Conclusions.*

(1) The characteristic feature of chronic rat plague as described in the foregoing account is the presence of circumscribed abscesses containing plague bacilli in rats caught alive, the animals usually showing no other lesions nor signs of ill-health. No bacilli were seen on microscopical examination of the heart-blood and of the spleen tissue in any of the rats. The bacilli in the great majority of the cases were virulent.

(2) We have grouped the 45 rats conforming to this description which were found during the year's investigation in the Punjab into two classes, one group including those in which the lesions were situated in the abdominal viscera, and the other group including those in which the abscesses were found in regions occupied by peripheral lymphatic glands.

(3) Lesions of the viscera were found principally in the spleen and in the mesentery, while the submaxillary group are most frequently affected among the lymphatic glands.

(4) The peripheral type was observed chiefly during the decline of the epizootic, while the visceral type predominated in the off-season.

(5) In Bombay, only one chronic plague rat was met with out of 17,000 plague-infected rats. In Kasel, 9% of all the rats which were proved plague infected had the chronic disease, while in Dhand the proportion was as high as 28%. With our present knowledge we can advance no adequate explanation of these facts.

(6) We have no direct evidence that chronic plague, as it occurs in the Punjab villages, possesses any significance in the seasonal recurrence amongst the rats of the infection in an acute form, nor is any evidence available which excludes this possibility.

## XX. A NOTE ON MAN AS A HOST OF THE INDIAN RAT FLEA (*P. CHEOPIS*).

Experiments already published (vol. VI, p. 435), and since repeated with similar results, afford ample confirmation of the observations of Simond (1898) and of Gauthier and Raybaud (1902, 1903) that plague may be transmitted from rat to rat by the agency of rat fleas. The direct experiment to show that plague can be conveyed in the same way from rat to man can never be made, and the inferential transference of the results of the experiments on rats to the spread of human plague has been not a little opposed, notably by Galli-Valerio (1900, 1903), on the ground that rat fleas do not bite men. This may be true of the fleas (*C. fuscatus* and *T. musculi*) commonly found on rats in Western Europe, but Gauthier and Raybaud (1902, 1903) in Marseilles, and Tidswell (1903) in Sydney, obtained direct evidence to the contrary with regard to *P. pallidus* (= *P. cheopis*), and Liston (1905) found that *P. cheopis* in Bombay readily attacks man during the plague season.

We have made many observations which show that *P. cheopis* will make use of man as a host, and may be captured in large numbers on men in houses infested with rat fleas.

### I. *Observations made in the laboratory.*

During the last year or more we have had occasion to make a large number of experiments on the transmission of plague from animal to animal by means of the rat flea. We have also carried out many experiments on the breeding of these fleas at different seasons of the year. During the course of these observations we have often noticed that, if a man's hand is put into a cage containing rat fleas, the fleas will jump on to the hand, and, if given time, will feed on it. This they will do the more readily, if they have been left without their natural food, a rat, for 24 hours or longer. We

have also made the observation that, if the fleas in the cage are abundant and the natural food supply limited, a few fleas will feed on a man's hand even in the presence of a rat. Having, therefore, demonstrated that rat fleas would feed on man, we next proceeded to ascertain if they could be kept alive on this diet for any length of time. The following was the method employed:—about 40 fleas, caught on healthy Bombay rats, were placed in a wide-mouthed jar, which had a little sand at the bottom. Twice daily a man's hand and forearm were introduced into the jar and left in for 15 minutes each time. Fleas which crawled up the forearm were gently pushed back before they could escape. It was observed that the fleas bit readily and the man himself was soon cognisant of the fact. About every ten days the sand was removed and fresh sand substituted, so that multiplication of the original fleas by breeding was excluded. Five experiments in all were made in this way, with the following result.

*Experiment 1.* One flea was found alive on the 24th day.

*Experiment 2.* One flea was found alive on the 9th day. In this experiment the sand had not been washed, and there was present a fine dust, which by blocking the tracheal openings was probably prejudicial to the lives of the fleas. They were often observed to be dusted over with this material.

*Experiment 3.* One flea was found alive on the 25th day.

*Experiment 4.* One flea was found alive on the 25th day.

*Experiment 5.* One flea was found alive on the 27th day.

From the above experiments it is seen that we were able to keep rat fleas alive for nearly four weeks by feeding them on human blood. There was a considerable mortality during this time, but the main facts stand out, (*a*) that they fed readily on man, and (*b*) that some of them were still alive after 25 days.

Rat fleas kept under similar circumstances, but without food, never survived longer than one week.

## II. *Observations in the course of the godown experiments.*

We have previously (vol. VI. p. 450) detailed a number of experiments made in godowns or cabins, some of which were infested with rat fleas. The present observations were made mostly during some recent experiments similar to those already described.

In the course of these experiments men had to enter the godowns

for the purpose of removing dead animals and for the purpose of feeding those which remained alive. We have on many occasions caught fleas on the legs<sup>1</sup> of these men, especially if, as happened in some instances, the godown had been empty for some days. It was also noted that if guinea-pigs were present it was still possible to obtain a few fleas on the legs of those entering the godowns. Several observations have been made, in which the fleas which were caught after the man had been in the godown for a given time were enumerated. Three of these observations will serve as examples:—

1. Godown 1 contained abundant rat fleas. On 3. XII. 06 a man entered the godown to remove some cages containing monkeys which had been kept inside for two days. He entered the godown four times, and 44 fleas in all were caught on his legs; 25 the first time he came out, 10 the second time, 3 the third time, and 6 the fourth time. Each time he waited in the godown no longer than was required to pick up and remove the cage.

2. Godown 1 contained abundant rat fleas. On 19. I. 07 a man entered the godown four times to remove some cages containing monkeys, which cages had been inside for two days. The first time he entered he was in the godown for half a minute, and on his coming out 19 fleas were taken on him; the second time he was inside for quarter of a minute and 5 fleas were taken on him; the third time he was again inside for quarter of a minute and 5 fleas were taken on him; the fourth time he was inside for 20 seconds and 1 flea was taken on him. Altogether he was in the godown for about  $1\frac{1}{4}$  minutes and 30 fleas in all were taken from his legs.

3. An exactly similar observation to the above was made on 25. I. 07, when 15 fleas were taken on the man<sup>2</sup>.

### III. *Observations made in houses in Bombay.*

During the epidemic of 1906 we had occasion to make numerous observations, as has been already described (vol. VI. p. 467), in houses in Bombay which were presumably plague infected. In the course of these observations our assistants and ourselves had to enter the houses for the purpose of placing animals therein. We have on many occasions

<sup>1</sup> It is to be noted that the men had always their legs bare.

<sup>2</sup> It may be noted in passing that as these godowns were infected with plague the men, whose duty it was to enter them, had been previously well immunised with Haffkine's prophylactic. None of them contracted the disease.



caught rat fleas on our own persons, as well as on those of the attendants. We have in some instances made definite enumerations of the fleas caught and have at the same time noted the species carefully.

280, *De Lisle Road*. We have recorded already (vol. vi. p. 480) several experiments carried out in this building, in which animals protected from fleas escaped infection, while those which were unprotected developed plague. The building was a long corrugated iron shed with earthen floor and divided into small rooms by partitions. Many plague-infected rats were found, while in some of the rooms plague cases had occurred. The building was, in short, unusually severely stricken with plague, and had been evacuated when the present observations were made.

(1) *April 17th*. 40 fleas were caught on a man who went into one of the rooms for a short time. They were all *P. irritans*.

(2) *April 18th*. 113 fleas were caught on a man who entered one of the rooms. The species were as follows:—*P. irritans* 55, *P. cheopis* 51, *P. felis* 7.

(3) *April 19th*. 76 fleas were caught on a man who entered one of the rooms for a short time. The species were as follows:—*P. irritans* 40, *P. cheopis* 34, *P. felis* 2.

(4) *April 20th*. 80 fleas were caught on a man who entered one of the rooms. The species were as follows:—*P. irritans* 18, *P. cheopis* 60, *P. felis* 2.

Thus, in three out of four rooms of this chawl, which was badly infected, abundant rat fleas were taken on the legs of men who entered the rooms only for a short time.

### *Summary and Conclusions.*

We have shown that in the laboratory the rat flea, *P. cheopis*, will readily bite man. When very numerous it will bite man even in the presence of its natural host. We have been able to keep this species of flea alive for more than three weeks by feeding it on man alone.

In the course of some experiments in godowns which were infested with *P. cheopis* alone, we have often taken fleas in considerable numbers on the legs of men who have entered the godowns for a short time.

In a building in Bombay, in which there had been a severe rat mortality, proved to be due to plague, we have taken rat fleas in large

numbers on the legs of men who entered some of the rooms in this building even for a short time.

We can conclude, therefore, that the rat flea, *P. cheopis*, under certain circumstances, is attracted by man, and will readily bite and feed on him.

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## THE BACTERIOLOGICAL EXAMINATION OF SURFACE WELLS.

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THE results of the bacteriological examination of surface wells are more difficult to interpret, in relation to the determination of pollution, than are those obtained with any other class of natural waters.

The influence of immediate local conditions on the bacteriological findings for these water supplies is always considerable and must be taken into account. At the same time the proper determination of the significance of the results of chemical and bacteriological examinations of surface wells is very important, since a considerable portion of the rural population is dependent for its entire drinking water upon shallow wells.

My experience leads me to conclude that chemical analysis is particularly unreliable for this class of waters.

Topographical investigation is of the utmost importance but alone it frequently does not enable a decided reliable opinion to be given whether contamination is, or is not, present, or possible.

The published results of the bacteriological examinations of surface wells are not numerous, while there are none, with which I am acquainted, in which the results are considered in the light of a topographical knowledge of the surroundings of the wells.

The wells included in this paper were all personally examined and the source of the water and its liability to pollution investigated. All but one of them are in the Borough of Colchester, which is very extensive in area and in large part rural. The samples were collected over a space of 3 years, but the great majority within the last 15 months as part of a special investigation. All the samples were personally collected except a few of the duplicate samples from wells already examined, and these were collected with great care and all by

## 478 *Bacteriological Examination of Surface Wells*

the same inspector. In every case the examination was started within two and a half hours of collection, while for most the interval was much shorter than this.

Nutrient gelatin media of a +1 per cent. reaction was used for cultures placed at 20—21°C., the colonies being counted after 3 days' incubation, for practically all the samples. Nutrient agar, also of a +1 per cent. reaction, was used for cultures placed at 37°C., the colonies being counted after 42 to 48 hours' incubation.

For the *Bacillus coli* estimations some variations of method were employed, but for the great majority of the samples a procedure combining the use of the neutral-red and bile-salt broth methods was used. In every case some of the dilutions were plated, the *B. coli*, or coli-like organisms, isolated, if present, and their characters determined in pure culture.

The *streptococci* enumerations were made by adding varying quantities of the sample, e.g. 0.1, 1, 10 c.c. to tubes of glucose neutral-red broth, while for the larger amount of water a tube of four times strength glucose neutral-red broth was added to this quantity of water in the collecting bottle. The different mixtures were incubated at 37°C. for 48 hours and were then examined, in hanging drop preparations, for streptococci in chains. In only a few instances were the streptococci isolated and their characters determined, but in all doubtful cases the fluid was stained and examined. Only cocci which occurred in definite chains were regarded as streptococci and so recorded.

The results here recorded are based upon the examination of 86 samples of surface well waters obtained from 50 different surface wells.

29 wells examined once	= 29 samples.
12 " " twice	= 24 "
3 " " three times	= 9 "
6 " " four	" = 24 "

The results and details of an examination upon the effects of a soil environment upon *B. coli*, are also included and considered.

### PART I.

#### *The bacterial content of the wells.*

The wells and the bacteriological results can be considered in 3 series; from the point of view of the topographical surroundings of the wells.

*Series A.* Wells which on careful inspection were considered to be free from any risk of contamination.

*Series B.* Wells which when examined showed obvious possibilities of more or less direct pollution.

*Series C.* Wells which when examined showed no gross definite evidence of pollution, but which from their proximity to houses, or from being surrounded by manured land, were considered to be probably liable to pollution. In this series are included a number of samples from wells situated in the rear premises of houses in the midst of the town itself, and frequently in populous neighbourhoods. These wells were all covered over and provided with a pump, and for many of them the exact locality of the well could not, with certainty, be ascertained. The houses were all drained and supplied with water-closets, there being no privies or known cesspools in their neighbourhood. The wells were for the most part quite near the houses.

The wells, with two exceptions subsequently indicated, were all, as far as they could be seen, of the same type, i.e. brick lined, but not rendered impervious in any way. Their depth varied greatly in the different parts of the Borough.

The results of the examination of *Series A* wells are given in Table I. This table shows that the bacteriological findings were quite in accord with the topographical inspections, although the latter were noted quite independently, and indeed were recorded at the time the samples were collected. The classification into the three groups was therefore made before any bacteriological results were available, in this way eliminating any unconscious influence upon the topographical classification. Only 8 wells (10 samples) could be classed in Series A.

Excluding one well, the well from which samples 4, 18, and 71 were obtained, all the samples were free from *B. coli* and streptococci in 50 c.c. Larger amounts were not examined.

Bacteriologically I should therefore certainly pass these waters as satisfactory.

The numerical counts are interesting. The enumerations at 37° C. are surprisingly low, considering the possibilities of soil organisms being washed into the wells. The gelatin counts show that enumeration at 20—21° C. is practically valueless for surface wells. Disturbance of the water may produce an enormous increase in the number of these organisms growing on plates, and this without the addition of harmful organisms. This is well shown in sample No. 1.



## 480 *Bacteriological Examination of Surface Wells*

The well from which samples 4, 18 and 71 were collected is a well which is properly protected from pollution, and is rendered impervious to a considerable depth. It is covered over with a wooden cover provided with a lock and key. The water is pumped by a wind pump up to a tank and then distributed. Sample No. 4 was quite satisfactory. No. 18 is less satisfactory, but is to be accounted for by the disturbance two months previously, when fresh feeders were constructed. Sample 71 is also not perfectly satisfactory, and a slight but sufficient surface contamination due to carelessness when the cover was unlocked was discovered which probably accounts for this.

The results of the examination of *Series B.* wells, shown in Table II, are in marked contrast. In this series 11 wells are included, from which 22 samples were collected. For the majority of the samples, but not all, the bacteriological findings would be sufficient to condemn the supplies, apart from the knowledge of topographical conditions.

Table II shows several points of interest. In the first place in several instances it will be noticed that for the same well at one time *excretal B. coli* were isolated, while on other occasions no such typical organisms were found, but only bacilli which differed more or less widely from the typical *B. coli*. It is probable that, for at least some of these samples, true *B. coli* were present but were not isolated. This is a difficulty not infrequently met with in this class of waters. If these aberrant coli-like organisms are altogether neglected, as some bacteriologists seem to hold should be the case, and the purity of the samples are considered entirely from a *B. coli* standpoint, and from one for which only quite typical organisms are considered, several of the samples from undoubtedly polluted sources would have to be passed as showing no evidence of pollution. Samples 6, 75, 31, 85, 55 illustrate this point.

In the second place the question of the amount of rainfall previous to the sampling seems to play a considerable part in modifying the bacteriological findings.

For example, the well from which samples 31, 37, 48 and 73 were collected was a covered well provided with a pump. The covering over the well was, however, very defective, open joints existing between the stone slab immediately over the well, and the smaller stone slabs surrounding it. The well stood in a yard about 30 feet from the house, while 12 feet away in the opposite direction was a privy and urinal, and next to them a quantity of pigs were kept.

The depth of the well was not actually measured, but from a knowledge of the wells in the immediate neighbourhood it can be stated with considerable certainty that the level of the water at the time of the examinations was not less than 30 feet. The well had been there certainly for more than twenty years and the inside was not rendered impervious or protected in any way.



## 482 *Bacteriological Examination of Surface Wells*

This well was obviously liable to marked pollution. Samples 31 and 37 collected within a week of one another and when there had previously been but very little rain for many weeks, show this pollution, while sample No. 73 also shows it very clearly. Sample 48 on the other hand taken after considerable rainfall was very different and did not show pollution. The water was only pumped to waste for 2 to 3 minutes before this sample was collected; probably if prolonged pumping had taken place the contamination would have been evidenced bacteriologically.

Samples 6, 24, 41 and 75 show a somewhat similar condition of affairs. They are from a similar well in the same village.

Samples 49 and 57 are of interest. This well supplies four houses. It is an open hole, rather than a well, about 4 feet deep with a wooden boxing round it and which only partially covers it. It is in a hollow and the water contained in it is apparently derived from a spring.

Behind and rising 6 feet above it is a heavily manured field. On one side about 10 feet away is an ordinary public road, and from the road a cutting conducts all the surface washings from the road direct into the well. With considerable rainfall the washings from the road must run down into the well, but when both the samples were collected the channel from the road was quite dry, and there had not been any rainfall for some time previously. The manured field behind the well is also a source of pollution. With only moderate rainfall the manurial organisms would have to pass through the 6 feet of soil before they could gain access to the water, but with heavy rain the washings would certainly overflow the 6 foot bank which rises steeply up from the well and so gain direct access to the water.

Here the condition of things showed that marked surface pollution was inevitable, but mainly dependent upon the rainfall. Both samples were collected when there had previously been but little rain and showed no streptococci or *B. coli* in either sample, much, I must confess, to my surprise.

This water, really a spring water, is a good illustration of a water supply which would be at once condemned on inspection but the dangers of which were not shown by the two samples bacteriologically examined. I have not found such a condition of affairs one at all commonly met with.

The 37°C. and 20°C. enumerations show extensive variations and cannot be said to be of much assistance in arriving at an opinion. It will be noticed however that the counts at 37°C. are in general high and show a marked difference from those recorded in Table I.

On the whole the *B. coli* and streptococci results show marked agreement. The streptococci results are much more uniform for the same well than are the *B. coli* findings.

The majority of the samples belong to *Series C* and it is for these that bacteriological assistance is most required.

The wells of this series form several distinct groups.

Group  $\alpha$  forms the largest class. It consists of draw wells in country districts and villages. All the wells were situated in gardens or patches of ground attached to the houses but often at some little

distance away from the houses. The ground around them was usually cultivated and manured. As a rule they were fairly deep, and without exception they were brick lined without any impervious backing or rendering of the surface in any way. They were either completely open or partially protected by a matchboard covering provided with a hinged door. The brickwork of the well, for the great majority, was continued above the ground effectively preventing the direct access of surface waters. The water was collected in ordinary pails lowered and drawn up by the usual chain and handle.

Wells similarly situated and equally unprotected are commonly met with all over the rural parts of the country. The mouth of the well being unprotected pollution from the pail used, and in other ways, is to some extent inevitable, but apart from this, organisms would have to filter through some depth of earth before they could gain access to the water.

The results of the examination of a number of such wells are shown in Table III.

With these results may be compared those shown in Table IV. This table shows the results of the examination of wells (Group  $\beta$ ) quite similarly situated to those of Group  $\alpha$ , the essential difference being that they were provided with a pump, the well being otherwise completely covered over and protected from surface pollution.

The comparison between Tables III and IV is very instructive. For example the pump from which samples 29, 36, 47, 82 were collected is in the same village and quite near to the wells from which samples 5, 22, 39, 70 and 7, 23, 38, 79 and 8, 28, 74 were obtained. In other words they have the same subsoil water. Sample No. 30 was from another pump in the same village.

The first-named well (No. 29 etc.) had been much more recently constructed than the others and was in a small grass field, the ground round was not manured. Consequently this well had advantages over the others in addition to being covered, but No. 30 was quite comparable to the others.

The results show what an extensive part surface pollution plays in altering the results of the examination, and probably if many of these open wells had been properly covered in and provided with a pump so that all surface pollution is avoided the bacteriological results would not have shown extensive pollution.

It must be remembered that the soil contamination in these rural areas is comparatively slight as regards human pollution, although

TABLE III.

No. of sample	Date of examination	No. of organisms per c.c. developing at		'Excretal' <i>E. coli</i> in				Coli-like organisms isolated from				Streptococci in				Class of Well	Remarks
		37° C.	20° C.	0.1	1	10	40 c.c.	0.1	1	10	40 c.c.	1	10	40 c.c.	1		
2	Feb. '04	1500	5000	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
3	March '04	82	5000	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
60	April '06	18	14500	-	-	-	+	-	+	+	+	-	-	-	-	Draw	
5	April '04	186	1700	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
22	Oct. 4, '05	1130	2720	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
39	Oct. 26, '05	340	8600	-	-	+	+	+	+	+	+	-	-	-	-	Draw	
78	Sept. '06	450	...	+	+	+	+	+	+	+	+	-	-	-	-	Draw	
7	April '04	206	2600	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
23	Oct. 4, '05	900	2800	-	-	+	+	+	+	+	+	-	-	-	-	Draw	
38	Oct. 18, '05	2080	13700	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
79	Sept. '06	2320	...	+	+	+	+	+	+	+	+	-	-	-	-	Draw	
8	April, '04	44	1600	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
28	Oct. '05	110	5170	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
74	Aug. '06	370	...	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
9	May '04	...	2500	-	+	+	+	+	+	+	+	-	-	-	-	Open; draw	Recently cleaned out.
16	Feb. '05	220	over 50000	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
20	July, '05	30	...	-	+	+	+	+	+	+	+	-	-	-	-	"	
27	Oct. 9, '05	415	5460	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
35	Oct. 18, '05	645	13400	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
80	Sept. '06	3250	...	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
32	Oct. '05	305	14100	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
81	Sept. '06	950	...	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
33	Oct. '05	272	6440	-	-	-	+	+	+	+	+	-	-	-	-	Draw	
76	Aug. '06	1300	...	-	-	-	+	+	+	+	+	-	-	-	-	Draw	
34	Oct. '05	134	3220	+	+	+	+	+	+	+	+	-	-	-	-	Draw	
84	Sept. '06	296	...	-	+	+	+	+	+	+	+	-	-	-	-	Draw	
43	Nov. '05	27	3300	-	-	-	+	+	+	+	+	-	-	-	-	Draw	
62	April '06	165	14000	-	-	-	-	-	+	+	+	-	-	-	-	Draw	
15	Dec. '04	3420	over 50000	-	-	-	-	-	+	+	+	-	-	-	-	Protected	Recently disturbed.



TABLE IV.

No. of sample	Date of examination	No. of organisms per c.c. developing at		'Excretal' <i>E. coli</i> in				Coli-like organisms isolated from				Streptococci in				Class of Well	Remarks
		37° C.	20° C.	0.1	1	10	40 c.c.	0.1	1	10	40 c.c.	1	10	40 c.c.	1		
29	Oct. 9, '05	0	350	-	-	-	+	-	-	-	-	-	-	-	-	Pump	
36	Oct. 18, '05	5	530	-	-	-	-	-	+	-	-	-	-	-	-		
47	Feb. '06	4	530	-	-	-	-	-	-	-	-	-	-	-	-		
82	Sept. '06	6	...	-	-	-	-	-	-	-	-	-	-	-	-	Pump	
46	Nov. '05	9	2020	-	-	-	-	-	-	-	-	-	-	-	-		
30	Oct. '05	78	1060	-	-	-	-	-	-	-	-	-	-	-	-		
44	Nov. '05	20	4260	-	-	-	-	-	-	-	-	-	-	-	-	Pump	
63	April '06	5	505	-	-	-	-	-	-	-	-	-	-	-	-		
56	March '06	...	1170	-	-	-	-	-	-	-	-	-	-	-	-		
59	April '06	6	1330	-	-	-	-	-	+	+	+	-	-	-	-	Pump	
72	Aug. '06	12	...	-	-	-	+	-	-	-	-	-	-	-	-		
77	Nov. '06	143	5300	-	-	-	+	-	+	+	+	-	-	-	-		
86	Sept. '06	4	...	-	-	-	-	-	+	+	-	-	-	-	-	Pump	

Same well as No. 12 Table.

TABLE V.

No. of sample	Date of examination	No. of organisms per c.c. developing at		'Excretal' <i>E. coli</i> in				Coli-like organisms isolated from				Streptococci in				Class of Well	Remarks
		37° C.	20° C.	0.1	1	10	40 c.c.	0.1	1	10	40 c.c.	1	10	40 c.c.	1		
13	Dec. '04	30	480	-	-	-	+	-	-	-	-	-	-	-	-	Pump	
14	Dec. '04	0	40	-	-	-	-	-	-	-	-	-	-	-	-		
50	March '06	4	150	-	-	-	+	-	-	-	-	-	-	-	-		
68	April '06	8	700	-	-	-	-	-	+	-	-	-	-	-	-	Pump	
51	March '06	10	220	-	-	-	-	-	-	-	-	-	-	-	-		
52	March '06	9200	innumerable	-	-	-	+	-	-	-	-	-	-	-	-		
69	April '06	112	7700	-	-	-	-	-	+	-	-	-	-	-	-	Pump	
53	March '06	38	570	-	-	-	-	-	-	-	-	-	-	-	-		
64	April 11, '06	72	2300	-	-	-	+	-	+	+	+	-	-	-	-		
67	April 24, '06	16	1340	-	-	-	+	-	+	+	+	-	-	-	-	Pump	
65	April '06	38	5300	-	-	-	-	-	-	-	-	-	-	-	-		
66	April '06	26	5200	-	-	-	+	-	+	+	+	-	-	-	-		

sometimes the ground round, or at least near, the wells is extensively manured. As a rule, however, this does not extend up to within several yards of the well so that all the organisms applied to the surface would have to pass through a good many feet of soil before they could gain access to the water supply. The filtering power of the soil is great and frequently much underrated, and the results here recorded lend much support to the view that if such wells are properly covered and provided with pumps, and particularly if, in addition, they are rendered impervious to a depth of a few feet, they form, in really rural districts, a reasonably safe source of supply. A depth of 10 to 12 feet is sufficient for the well to be imperviously lined. Failing this a definite area round the well should be rigidly protected.

The difference between the open draw wells and the pump provided wells is clearly shown by the *B. coli* and streptococci results, but it is even more marked in the enumerations, especially the 37° C. determinations.

A comparison between the two samples 62 (Table III) and 64 (Table IV) brings this out very clearly. Both samples were collected the same day, the two wells are quite close to one another, and if proximity to houses and possible sources of pollution is a test of purity, No. 62 is the better sample. As regards *B. coli* and streptococci both are satisfactory, but the difference between the open well and the surface protected well with proper pump is very obvious for both the 37° and 20° enumerations.

Samples 12 and 46 both from the same well are very instructive.

Sample 12 (Table II) was collected Sept. 1904. When then seen the well was covered and provided with a pump, but the covering was defective and less than 10 feet away was a cesspool which was leaking into a field adjacent to the well. The well was a very shallow one and not rendered impervious in any way, but was lined with bricks. The well was condemned, and to meet my requirements was deepened, rendered impervious by a cement lining and a thick backing of clay, to a depth of 10 feet, while it was properly covered, so that surface water could not gain access. At the same time the drains were relaid and an impervious cesspool provided.

The second sample (No. 46, Table IV), collected November, 1905, about a year after the alterations, showed no *B. coli* or streptococci in 50 c.c. and may be considered satisfactory.

An entirely fresh source of drinking water was not available, but the above shows that it may be possible by suitable alterations to convert an initially highly contaminated source of supply into one which may be considered satisfactory and free from risk.

The chemical analysis, as far as it was carried out, for seven of these wells is given in Table VI. These wells were all in the same village. The chief interest of the figures is that the results do not show the

differences met with on bacteriological examination, or certainly not to anything like the same extent. Compare for example the chemical and bacteriological analyses of the wells from which samples 27 and 29 were respectively taken.

The remaining wells of *Series C* can be included in a third group—Group  $\gamma$ . This group consists of wells situated in the town itself and usually surrounded with houses. The houses supplied have for one reason or another avoided taking the public supply of the town, remaining content to use the old well water. Without exception they were covered and provided with pumps. The houses surrounding them were all drained and provided with water-closets, no privies or known cess-pools being in their neighbourhood. The soil round should therefore be free from pollution. They are all old wells and, although not examined, it can be assumed as almost certainly true that none of them would have their interior rendered at all impervious.

The position of this group of wells would certainly render them liable to suspicion on inspection, but if a properly constructed and tight drainage system is present, and the coverings are water-tight, such wells may really not be liable to dangerous pollution. Bacteriological examination is here particularly valuable to say whether they are actually being contaminated or not.

The results of the examination of a number of such wells are shown in Table V.

Samples 13 and 14 are taken from wells surrounded by houses which tap a considerable supply of water in the middle of the town of Colchester, water which finds an outcrop as a spring about a quarter of a mile away on the side of a narrow valley of considerable depth. It is evidently a pure supply as shown by sample 14 although it flows under a highly populated area.

Sample 13, a well quite close to No. 14, is rather less satisfactory, apparently because the water is stored in a large tank, and the sample was collected after storage in the tank.

Samples 50, 68, 51, 52 and 69 are from wells supplied with pumps, all of which tap this same underground water. On the whole, except No. 52 well, they are not unsatisfactory. For this well the contamination was probably from surface water gaining access.

Mere inspection of these wells is insufficient to determine if they are contaminated or not. Thus the well from which samples 64 and 67 were collected, was on topographical inspection certainly as favourably situated as wells 13, 14, 51, 53, etc. and it was impossible from inspection alone to say that it was contaminated. The bacteriological results were however conclusive, and the well was closed.

488 *Bacteriological Examination of Surface Wells*

TABLE VI.

*Chemical Analyses figures. Samples all collected Oct. 1905.*

Source of the samples	Physical characters (in 2 foot tube)	Reaction	In parts per 100,000					Sediment
			Chlorine	Total solids	Nitrogen as nitrites and nitrites	Saline ammonia	Albuminoid ammonia	
The same well as 27, 35, 80	Slightly turbid, colourless	Neutral	4.3	34	0.64	0.007	0.008	Abundant débris, some animalculae.
32, 81	Clear, colourless	Faintly acid	5.0	—	0.36	0.012	0.019	Slight, a few animalculae.
29, 36, 47, 82	Quite clear, colourless	Neutral	5.9	62	0.84	0.008	0.006	Nil.
7, 23, 38, 79	Somewhat turbid, colourless	Faintly acid	4.0	—	0.54	0.01	0.016	Considerable, some animalculae.
25, 40, 83	Somewhat turbid, yellow brown	Faintly acid	4.8	—	0.84	0.01	0.0196	Slight débris and a few animalculae.
5, 22, 39, 78	Turbid, slightly yellow	Faintly acid	4.7	—	0.59	0.008	0.013	Considerable. Vegetable débris, animalculae.
6, 24, 41, 75	Quite clear, colourless	Faintly acid	4.1	—	0.48	0.01	0.015	Slight. Nematode worms found.

TABLE VII.

	Unpolluted (Series A)	Obviously liable to pollution (Series B)	Doubtful (Series C)	Results stated as percentage			
				Total	Unpolluted	Obviously liable to pollution	Doubtful
Number of samples examined	10	22	54	86	—	—	—
'Excretal' <i>B. coli</i> present in 0.1 c.c.	0	4	3	7	0	18	6
„ „ „ 1.0 „	0	11	14	25	0	50	26
„ „ „ 10 „	0	12	20	32	0	55	37
„ „ „ 40 „	1	13	24	38	10	59	44
„ „ „ absent in 50 „	9	9	30	48	90	41	56
Streptococci present in 1 c.c.	0	6	13	19	0	27	24
„ „ „ 10 „	0	15	17	32	0	68	31
„ „ „ 40 „	1	17	26	44	10	77	48
„ „ „ absent in 50 „	9	5	28	42	90	23	52

Dealing with the results of all the analyses one fact stands out strikingly, and this is the close relationship between the *B. coli* and the streptococci results.

This is brought out in Table VII. For *Series A* the results are identical. For *Series B* they are very similar, while for *Series C* they

are almost identical. Thus for *Series C*, *B. coli* and streptococci are present in 1 c.c. in 26 and 24 per cent.; in 10 c.c. in 37 and 31 per cent., and in 40 c.c. in 44 and 48 per cent. respectively.

This table also enables a rough comparison between the topographical and bacteriological results to be made.

## PART II.

### *The characters of the coliform organisms isolated from surface wells with a consideration of their significance and origin.*

Excluding a large number of organisms isolated but only incompletely investigated, 86 organisms were isolated and their characters determined to the extent shown in Table VIII. They can be conveniently classified into the following groups:

Group	Number isolated	Percentage
1. 'Excretal' <i>B. coli</i>	23	29.5
2. 'Excretal' <i>B. coli</i> modified as indicated	20	25.6
3. Modified <i>B. coli</i> -like organisms	10	12.8
4. Non-lactose fermenters	17	21.8
5. Considerably variant organisms	8	10.3
6. Non-glucose fermenters	8	—

The percentages are calculated after exclusion of the non-glucose fermenters, as only a few of these were worked out, generally for some special reason. The percentages refer only to glucose fermenters.

In Groups 1 and 2 the term *excretal B. coli* is used in the sense suggested by me in a previous paper<sup>1</sup> for an organism which is a short bacillus with rounded ends, producing a translucent non-corrugated appearance on gelatin slope; non-liquefaction of gelatin (2 weeks); permanent acid production in litmus milk with clotting of the milk within two weeks; lactose and glucose fermentation with both acid and gas production; a positive neutral-red reaction, and production of indol. Only 20 organisms conformed in every respect to the above characters, but with these may be included two organisms which were typical except that the neutral-red reaction was only partial, and a third which produced a rather thicker and whiter growth on the gelatin slope but was otherwise typical.

<sup>1</sup> Savage, W. G. (11. 1905). "The Characters of the *Bacillus coli* as an Indicator of Excretal Contamination," *Lancet*, vol. 1, p. 284.



TABLE VIII.

*Characters of the organisms isolated.*

Source. No. Sample	Mo- turity	Gelatin slope	Liquefaction of gelatin	Litmus milk			Coagulation	Time in days taken to co- agulate milk	Lactose fermenta- tion		Glucose fermentation	Saccharose fermentation	Dinitite fermentation	Indol fermentation	Neutral-red reaction	Starch fermentation	Remarks
									Present	Rate							
1	1	Yellow growth	+	+	+	+	+	6	+	ss	+	-	+	+	+	+	Liquefies gelatin slowly (4 weeks).
2	2	Smooth bluish translucent growth	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	" " (2 months).
3	3	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
4	6	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
5	8	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
6	9	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
7	70	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
8	12	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
9	13	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
10	15	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
11	18	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
12	19	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
13	20	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
14	22	Yellow slightly wrinkled growth	+	+	+	+	+	6	+	ac	+	-	+	+	+	+	Liquefies gelatin only after 3 months. Atypical gelatin colonies.
15	22	Yellow semi-translucent growth	+	+	+	+	+	7	+	ss	+	ac	+	+	+	+	Liquefies gelatin only after 4 months.
16	23	Smooth bluish translucent growth	+	+	+	+	+	3	+	r	+	-	+	+	+	+	"
17	24	"	+	+	+	+	+	6	+	ac	+	-	+	+	+	+	"
18	24	"	+	+	+	+	+	9	+	r	+	-	+	+	+	+	"
19	26	"	+	+	+	+	+	2	+	r	+	-	+	+	+	+	"
20	27	"	+	+	+	+	+	7	+	ss	+	-	+	+	+	+	"
21	28	"	+	+	+	+	+	-	+	ss	+	-	+	+	+	+	"
22	29	Smooth semi-translucent white growth	+	+	+	+	+	2	+	r	+	-	+	+	+	+	"
23	31	Translucent, yellow growth	+	+	+	+	+	7	+	slow	+	ac	+	+	+	+	"
24	31	Smooth bluish translucent growth	+	+	+	+	+	6	+	ac	+	-	+	+	+	+	"
25	31	Yellow, semi-translucent growth	+	+	+	+	+	13	+	slow	+	-	+	+	+	+	"
26	32	Smooth bluish translucent growth	+	+	+	+	+	7	+	r	+	-	+	+	+	+	"
27	33	"	+	+	+	+	+	7	+	ss	+	-	+	+	+	+	"
28	33	"	+	+	+	+	+	6	+	ss	+	-	+	+	+	+	"
29	34	"	+	+	+	+	+	7	+	ss	+	-	+	+	+	+	"
30	35	"	+	+	+	+	+	7	+	r	+	-	+	+	+	+	"
31	36	"	+	+	+	+	+	6	+	slow	+	ac	+	+	+	+	"
32	37	Transl. growth; later distinctly yellow	+	+	+	+	+	6	+	slow	+	-	+	+	+	+	"
33	37	Smooth bluish translucent growth	+	+	+	+	+	6	+	r	+	-	+	+	+	+	"
34	38	White, opaque growth	+	+	+	+	+	13	+	slow	+	-	+	+	+	+	"
35	38	"	+	+	+	+	+	1	+	slow	+	-	+	+	+	+	"
36	39	Smooth bluish translucent growth	+	+	+	+	+	2	+	r	+	-	+	+	+	+	"
37	40	"	+	+	+	+	+	1	+	r	+	-	+	+	+	+	"
38	41	"	+	+	+	+	+	2	+	r	+	-	+	+	+	+	"
39	42	"	+	+	+	+	+	10	+	r	+	-	+	+	+	+	"

Produces acid but no gas in mannite and matose media.



## 492 *Bacteriological Examination of Surface Wells*

As regards the 20 organisms of Group 2, for 11 of them the only difference was that no neutral-red reaction was given, for 5 the lactose fermentation was delayed and in several of these cases the gas produced was only slight in amount, for 3 the clotting of the milk was delayed, while for the remaining organism no indol was produced, but on retesting after a week's isolation indol was found to be present in slight amount. The variations from the 'excretal' type form are here very slight, and these two groups, forming rather over one half of the glucose fermenters, are included in Tables I to V as *excretal B. coli*.

Group 3 includes 10 organisms which differ in several particulars from *excretal B. coli*, for example in not coagulating milk. Group 4, that of non-lactose fermenters, includes 9 organisms which produced neither acid nor gas in lactose and 8 which produced a little acid but no gas. Group 5 includes organisms considerably modified, 6 of them producing a yellow growth on gelatin slopes while the other two, apparently identical organisms, slowly liquefied gelatin.

Only 29.5 per cent. were quite typical, and a characteristic feature of the bacteriology of surface well water is the large percentage of atypical organisms met with. In many of the samples from undoubtedly contaminated wells, no quite typical *B. coli* could be found, but organisms were isolated which deviated in several particulars from the accepted excretal type. In other cases the typical and the atypical coli were found side by side.

The origin and significance of these variant forms are of great interest and practical importance.

Three suppositions are possible as to their origin:—

(1) That they are derived from normal typical *B. coli* of faeces, which have become atypical by the loss of certain attributes owing to unfavourable environment. (2) That they are derived from identical atypical organisms in faeces or sewage, which, owing to greater hardiness and adaptability, have flourished better than the typical varieties, and have thus become relatively more abundant. (3) That they are true saprophytes, natural to water or soil, and are not of excretal origin.

The last supposition seems improbable, since if this were the true explanation we should expect to meet with such organisms equally in pure and impure soil, in pure water equally with polluted water. This, at least for the forms only slightly differing from *B. coli* as met with in the intestine, is not found to be the case, and these organisms are undoubtedly more prevalent in polluted than in pure

sources. Indeed they cannot be isolated from quite uncontaminated sources. It is highly probable therefore that their primitive origin is to be found in excretal matter.

If these organisms were derived from typical forms, two questions obviously arise: (*a*) Can such atypical organisms be converted into typical forms by artificial laboratory methods? (*b*) Can typical organisms, by cultivation in soil or water, be made to become atypical?

A summary of the experimental work of other observers in these two directions is given in my book on *The Bacteriological Examination of Water Supplies* (London, 1906), pages 145 to 153. Here I only wish to record and consider some additional experiments recently made in these directions.

As regards the first question (*a*) only two points were dealt with. The organisms which ferment lactose slowly might be considered to be typical *B. coli* which had lost the power to split up lactose vigorously owing to their saprophytic environment. The three organisms which were retested did not altogether bear out this view: No. 32 tested after 5 months in the laboratory (on gelatin slope) still showed only slow lactose fermentation. No. 44 retested after 2 months in the laboratory, showed now no gas production, but it formed acid. No. 28 retested after 6 months showed no change in the lactose medium, neither acid nor gas being formed.

The other character investigated was milk coagulating power:—

Nine organisms, which when isolated did not coagulate milk, were inoculated into litmus milk containing solid calcium carbonate. Four of the same organisms were also reinoculated into ordinary litmus milk. They were all incubated at 37°C. and were not disturbed or re-examined for a month. They were subsequently re-examined at intervals with the following results:

None of the 4 ordinary milk tubes showed any coagulation after one month and the two which were kept for two months also showed no coagulation. They all produced acid.

Six of the chalk-milk tubes, after one month, showed complete coagulation; 3 showed acid only. Of these 3, two, re-examined after 38 days, were completely clotted, while the other was alkaline and not coagulated. Coagulation was obtained therefore in 8 out of 9 instances in the chalk-milk.

These experiments would seem to show that the coagulation in these late cases is not due to the acid produced but rather to a ferment.

The calcium carbonate by neutralizing part of the acid produced allowed the organism to continue its growth until a sufficient amount of ferment was produced to coagulate the milk. This hypothesis is quite in accord with results obtained by me in 1904<sup>1</sup>, but I hope again to obtain the results with other strains of this organism.

As regards the second question (*b*) more extended work was carried out. It is obvious that sojourn of *B. coli* is likely to be more prolonged in soil than in water, and if alteration of character is to be traced it is more likely to be met with soil as the saprophytic environment than if water is the medium selected. Houston watered soil with sewage, but few experiments have been carried out with typical *B. coli* added to non-sterile soil and under quite natural conditions.

In the experiments carried out by me a number of carefully selected *B. coli* strains were added to natural soils and their fate studied through prolonged periods.

Two separate series of experiments were carried out with two different patches of soil which were free from *B. coli*. The patches were formed by removing the covering turf with a sterile spade, each was about 18 × 18 inches. The two soil areas were fairly near one another and consisted of a brown sandy soil, which had not been manured for many years, certainly not within 3 years. It was ordinary grass pasture land. Samples of the soil were removed by a sterile spatula from the surface from time to time, placed in sterile bottles and at once transmitted to the laboratory and there examined without delay. All the samples were personally collected by the writer.

#### *Experimental Patch, A.*

Three preliminary samples were collected before inoculation, *i.e.* on March 26th and 28th and April 4th, 1906. On March 26th two organisms *P*<sub>1</sub> and *P*<sub>2</sub> were isolated, but on the occasion of the other two examinations no organisms at all like *B. coli* were isolated although as much as 3 grammes of soil were examined on each occasion. *P*<sub>1</sub> and *P*<sub>2</sub> certainly were not *B. coli* (their characters are dealt with later on) so that it may be said with considerable confidence that *B. coli* was absent from this experimental soil.

On April 9th, the patch was watered with a mixture of *B. coli* races. For this purpose five strains of *B. coli*, obtained, two from polluted soil and three from cow manure, and one strain of *Bacterium lactis aerogenes* (derived from milk), were subcultivated on gelatin slope. From this slope each was inoculated into

<sup>1</sup> Savage, W. G. (1904). "The coagulation of milk by *Bacillus coli communis*," *Journ. of Path. and Bacteriol.*, vol. x. p. 90.



broth and 1 c.c. of the two days' broth culture of each was added to a litre flask of sterile water.

On April 9th this mixture was distributed over the patch. The six organisms had their characters re-determined immediately before being selected for the inoculation. They were all completely typical, and indeed were selected on this account.

Also the five *B. coli* strains from their action on saccharose and dulcitol could be divided into three groups. Their characters are given in Table IX. The five coli strains are  $S_1 S_2 S_3 S_4 S_5$  and the aerogenes  $S_6$ . The patches were left exposed and under perfectly natural conditions throughout, except that Patch A, between 9th and 19th April, received daily a quart of sterile water, since the weather was extremely dry.

On April 19th three samples were collected from Patch A. Ten organisms were isolated and their characters determined from the three different soils. Of these organisms four proved to be *B. coli* and with characters quite unchanged. The other six were all *Bact. aerogenes* and were quite unchanged from the original except that five now gave no neutral-red reaction and the other a partial reaction.

For the actual isolation of these organisms and for the examination of other samples, plates of Drigalski-Conradi medium and glucose neutral-red bilesalt agar were employed. In all cases the subcultivations were made in the first place into glucose neutral-red broth. If this was not fermented the organisms were not further examined, so that this investigation does not deal with organisms which have lost (if such be the case) the power to ferment glucose.

On May 9th (one month from seeding), three further soil samples from different parts of the patch were collected. In the same way 13 organisms were fully investigated. Of these two were *B. coli*. They both were perfectly typical. The remaining 11 were *Bact. aerogenes*. They were quite typical and like the original strain, except that six gave no neutral-red reaction and one a partial reaction. The aerogenes organism had evidently greatly outgrown the other five strains added.

On June 19th (ten weeks from seeding) three additional samples were collected. Only six of the isolated organisms fermented glucose. Of these two were *B. coli* and four *Bact. aerogenes*, although as far as possible *B. coli* colonies were selected for subcultivation.

Both the *B. coli* subcultivations isolated showed some deviation from the original forms. They belonged to separate strains (*a* and *b*), since one fermented dulcitol, but not saccharose, while the second fermented both these substances. The one (*a*) only differed from *B. coli* in that it fermented lactose but slowly and to a slight extent. Instead of producing at least 1 inch of gas in the inner tube within 24 to 48 hours, this organism after 48 hours formed  $\frac{1}{2}$  inch gas and acid and after a week's incubation at 37° C. not more than  $\frac{1}{2}$  inch of gas was produced. The other (*b*) *B. coli* strain in its action towards lactose was identical, producing, if anything, even less gas. This organism also exhibited no motility (24 hours' gelatin slope growth in broth in hanging-drop preparation) while the neutral-red reaction was incomplete.

The four aerogenes organisms were typical, except that one gave only a partial neutral-red reaction and the other three no trace of reaction.

On June 23rd, one week later, fresh samples were collected. Although 18 different organisms were subcultivated only five fermented glucose, and all these were *Bact. aerogenes*.

TABLE IX.

Number	Source	Motility	Gelatin slope		Liquefaction of gelatin	Litmus milk Acid and clot within two days	Lactose fermentation rate		Glucose	Saccharose	Dulcife	Indol	Neutral red reaction	Starch
S <sub>1</sub>	Cow excreta	+	Smooth bluish translucent growth		—		+	r	+	+	+	+	+	
S <sub>2</sub>	Soil	+	" "	" "	—	" "	+	r	+	+	+	+	+	
S <sub>3</sub>	"	+	" "	" "	—	" "	+	r	+	+	—	+	+	
S <sub>4</sub>	Cow excreta	+	" "	" "	—	" "	+	r	+	—	+	+	+	
S <sub>5</sub>	" "	+	" "	" "	—	" "	+	r	+	—	+	+	+	
S <sub>6</sub>	Milk	—	Thick, white growth		—	" "	+	r	+	+	—	+	+	+
S <sub>7</sub>	Cow excreta	+	Smooth bluish translucent growth		—	" "	+	r	+	+	+	+	p	
S <sub>8</sub>	Soil	+	" "	" "	—	" "	+	r	+	+	+	+	p	
S <sub>9</sub>	Surface well	+	" "	" "	—	" "	+	m	+	—	+	+	p	
S <sub>10</sub>	Soil	+	" "	" "	—	" "	+	r	+	—	+	+	p	
S <sub>11</sub>	Surface well	+	" "	" "	—	" "	+	r	+	+	+	+	—	
P <sub>1</sub>	Soil	+	" "	" "	—	Acid no clot	—	—	+	—	—	—	—	
P <sub>2</sub>	"	+	" "	" "	—	" "	+	slow	—	—	—	—	—	

r = rapid gas production.

m = moderate and not abundant gas production.

p = partial neutral-red reaction.

All five fermented lactose slowly and with little gas production. Four produced only a partial neutral-red reaction. One produced no indol. These particular strains when re-examined after being kept on gelatin slope for two months gave still a delayed lactose fermentation, yielding practically no gas after two days but a normal amount after a week, while the strain which gave no indol, now produced it in large amount.

On Oct. 19th, the last sample was collected, *i.e.*  $6\frac{1}{2}$  months from the original seeding. Very few glucose fermenters could be isolated, only seven organisms being found which fermented glucose. No glucose fermenters were present in 0.1 gramme.

These seven organisms were all identical. They differed widely from *B. coli*. They agreed in that the growth on the gelatin slope was typical, they were actively motile coli-like bacilli, produced indol, and rapidly fermented glucose. Neither dulcitol nor saccharose was fermented.

The points of difference were: no trace of neutral-red reaction was produced; although acid was formed in litmus milk no coagulation took place even after three weeks' incubation; lactose was only slightly fermented. In all acid was produced but either no gas or only a bubble or two.

The point at once arises, are these organisms altered *B. coli*?

The patch was protected from artificial contamination, but to be certain that they were not derived from adventitious pollution the surface soil just by the side of Patch A was examined, but no organisms of this or the *B. coli* group were found in as much as 3 grammes of it.

It may be assumed that these organisms either were derived from the *B. coli* (and aerogenes) strains added or were derived from organisms present in the soil at the time of inoculation. The two organisms  $P_1$  and  $P_2$  were isolated from the patch before inoculation. Their characters are given in Table IX. The only differences from the above are as regards glucose and lactose fermentation. Thus  $P_1$  differs only in that there is no trace of lactose fermentation.  $P_2$  differs only in that there is slow but considerable gas production in lactose media, while glucose is not at all fermented.

Two of the above organisms were re-investigated as regards their action on lactose after subcultivation in broth, gelatin slope, and broth and then into lactose media. They only fermented lactose with acid but no gas production, thus making them nearly indistinguishable from  $P_1$ .

In view of these facts it is not justifiable to say that these atypical organisms were derived from the typical *B. coli* with which the soil was inoculated.

Apparently all the *B. coli* had died out or become non-glucose fermenters.

*Experimental Patch, B.*

This patch was similar in character to Patch A. No *B. coli* or coli-like organisms were found in it before inoculation.

On July 8th, 1906, the patch was watered with five strains of *B. coli* ( $S_7 S_8 S_9 S_{10} S_{11}$  Table IX) in the same way as Patch A. They were all quite typical except that one produced no neutral-red reaction and the other four a partial reaction only. These organisms differed in two particulars from those used for Patch A in that, in the first place no *Bacterium aerogenes* was used, and in the second, several of the strains were organisms which showed some instability of character.

Thus  $S_9$  when isolated did not coagulate milk until after 11 days' incubation, while it then showed no motility. Also  $S_{11}$  when isolated yielded no indol and gave a complete neutral-red reaction.

On August 16th, the first samples after inoculation were collected (*i.e.* 40 days after seeding). Eight glucose fermenting organisms were studied. All failed to give a neutral-red reaction, two gave no indol and two gave a delayed time for milk coagulation (7 and 12 days respectively). Otherwise quite typical.

Sept. 13th further samples collected, 9½ weeks after inoculation. Nine glucose fermenters were studied. Most of them showed no neutral-red reaction, while for all but two milk coagulation was markedly delayed. The actual times taken to coagulate milk were 6, 22, 11, 21, 9, 21, 4, 20, 19 days respectively. They all fermented dulcitate like the originals and seven fermented saccharose and two not.

Part of the original batch of milk media tubes was used for the inoculations, while five were reinoculated into freshly prepared milk tubes with almost identical results.

Oct. 19th (after 15 weeks) a fresh sample was collected. No glucose fermenters could be isolated.

Oct. 25th (after 15½ weeks) a fresh sample collected. Six glucose fermenting organisms isolated. Except for absent or incomplete neutral-red reaction and a slightly delayed time for milk coagulation for two of the isolated forms, they were all completely typical.

It is to be noticed that all six fermented both dulcitate and saccharose, while the organism which coagulated milk in four days from the Sept. 13th sample also fermented both these substances. The alteration in coagulation time may possibly be explained by the fact that this strain had multiplied or happened only to be present where the actual sample was collected.

Nov. 12th (after 18 weeks) a final sample was collected. Very difficult to find any glucose fermenters at all and only three were met with. They varied as regards their action on neutral-red but otherwise were identical and quite typical. They all fermented both saccharose and dulcitate.

The results of the two series of soil investigations are not altogether easy to explain. In the first place they show that the neutral-red reaction is an unstable character for these organisms kept in soil. As regards the other characters indol producing power remained stable, the gelatin slope characters were unaltered and the motility was unimpaired.

In no case was definite loss of power to coagulate milk met with, but on one occasion organisms were observed which showed a marked delay in coagulating milk.

The delayed coagulation was noted in a number of instances in organisms isolated from the surface wells.

Also in several instances the organisms isolated had a diminished power of breaking up lactose. The action on lactose was delayed and in some instances only a little gas was produced.

This diminished lactose fermentating power has been already noticed as a characteristic of a number of the organisms isolated from the surface wells, while greater lactose splitting power could not be re-acquired for the three organisms retested. In an earlier paper<sup>1</sup> already referred to I drew attention to the existence of such organisms, and described the characters of 12 such bacilli. The fact that in these soil inoculation experiments such slow and diminished lactose fermenting bacilli were met with on several occasions and that they were undoubtedly derived from typical *B. coli* affords an additional support to the opinion I have expressed that these organisms are true *B. coli* altered by environment.

#### SUMMARY.

1. Numerical counts on gelatin media are of very limited value for surface well examinations and are quite useless for open draw-wells. The blood-heat enumeration is of use, but still of but limited value.

2. The influence of rainfall and local conditions generally is marked for this class of waters with regard to their bacterial content.

Whether the well is uncovered, or covered and provided with a pump has a very important influence upon the bacteriological results. For a good many wells which show very bad bacteriological findings the cause of pollution is frequently due to surface pollution rather than to pollution of the ground water through the soil. If these wells are properly protected they will become suitable sources of drinking supply.

3. The results obtained confirm the value of *B. coli* and streptococci estimations as the best tests by which to judge the freedom from pollution of such waters.

The streptococci results are of great value, only second in importance to the *B. coli* determinations. In numerical distribution these two

<sup>1</sup> See Footnote, p. 489.



## 500 *Bacteriological Examination of Surface Wells*

organisms closely agree. The presence of streptococci is more reliable as evidence to deduce pollution than their absence is to exclude it.

4. An opinion regarding the freedom of surface wells from pollution is best formed on the basis of combined topographical and bacteriological investigation.

5. Inspection alone is frequently quite insufficient for the purpose of determining if a well is contaminated and if it should be closed.

Of the 50 wells examined no less than 31 had to be classed as doubtful (Series C), topographical examination alone affording insufficient data for the expression of a decided opinion.

In view of the fact that pure water supplies are not easy to obtain in many rural districts, a water supply, which may have been in use for many years, cannot be condemned in the absence of clear evidence that it is unsatisfactory and dangerous.

6. In surface well waters a large proportion of the coli-like organisms isolated are atypical in one or more particulars.

7. Typical *B. coli* implanted into soil showed some alteration of character, but the changes were not extensive and no evidence was obtained that the widely aberrant organisms met with in different soils and waters ever represent typical *B. coli* altered by unfavourable environment.

8. Organisms closely allied to *B. coli*, but differing in one or more characters, possess significance as indicators of faecal contamination.

The more nearly the organism isolated resembles an "excretal" *B. coli* the greater is its significance as an indicator of pollution. Consequently the fewer the number required to condemn a sample water in which they occur. Stated as a working proposition, the more the characters of the coli-like organisms deviate from that which for convenience may be spoken of as the typical form, the greater the proportionate number of them required to condemn the water.

9. The presence of 'excretal' *B. coli* in 10 c.c. or less of a surface well water points to undesirable pollution and is sufficient to condemn the water.

## ON THE DANYSZ EFFECT WITH REFERENCE TO THE TOXIN-ANTITOXIN REACTION.

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THE investigation of the toxin-antitoxin reaction has been pursued during the past decade with great vigour, but the views advanced to account for the observations are, at present, highly divergent. It seems to me to be desirable, therefore, to review some recent and apparently important data, collected by Madsen and Walbum and calculated by Madsen and Arrhenius (1906) (1907), which bear directly on these interpretations.

One of the fundamental facts is that the reaction between toxin and antitoxin is practically independent of biological influences; in other words the interaction may, in many cases, be studied *in vitro*.

Ehrlich (1897) demonstrated this by experiments *in vivo* and *in vitro* for ricin and antiricin and on this basis, after numerous investigations of allied reactions, evolved his well-known "Side-Chain Hypothesis" as well as his "Spectra" for the constitution of toxins, *e.g.* of diphtheria toxin.

The striking and helpful "Side-Chain Hypothesis" is, as yet, without any worthy competitor, but Arrhenius and Madsen (1902) and Bordet (1903) and Landsteiner (1903) have advanced views that cast a new light upon the constitution of many of the best investigated toxins. Of the methods which have been acknowledged to be available in the attempt to arrive at a decision with regard to the relative value of the views advanced, that based upon the "Danysz Effect" seems to be one of the most important.

Danysz (1902) found that when ricin or diphtheria toxin was brought into contact with its corresponding antibody the degree of neutralisation depended upon the method adopted in preparing the mixtures, in the sense, that when the toxin was added to the antitoxin

in two fractions, a considerable time being allowed to elapse between the additions, the resultant mixture contained a much larger amount of free toxin than in the case when the total quantity of toxin was added at once to the antitoxin.

v. Dungern (1904) confirmed this result for diphtheria toxin and antitoxin, and attributed it to the action of a hitherto unknown substance in the toxin, viz. epitoxonoid, a view subsequently accepted by Ehrlich.

Sachs (1904) found similar relations to hold between tetanolysin, rennin and their corresponding antibodies, but not between cobra venom and its antivenene.

Craw (1905, III.) observed the "Danysz Effect" in mixtures of megatheriolysin and antilysin, and experimenting with "nearly neutral" fluids, *i.e.* such as had but a slight haemolytic effect, found the "Effect" greater with larger quantities of lysin.

Madsen and Arrhenius (1906) (1907) have given a very condensed account of their work and that of Walbum upon this theoretically very important phenomenon. They find the "Effect" greater when the antilysin is "in excess," but this difference seems to be less due to contradictory results than to our diverse definitions of a "neutral mixture" and to the nature of the materials studied. Madsen and Arrhenius ascribe the "Effect" to the production and presence of a modified antitoxin. It seems to me doubtful whether it is necessary to assume either a new constituent in the toxin or in the antitoxin; further, neither of these assumptions appears to me to be satisfactory, for the reasons given below.

In the first place the material, tetanolysin, used by Madsen and Arrhenius is unsuitable. Madsen (1899) himself showed that the haemolytic power of a 4% solution kept at 20° C. for five hours diminished by 50%. At 37° C. this effect is much greater amounting to 25% in one hour, as I have found on examining various brews.

In these experiments we are, therefore, dealing with an unnecessarily complicated phenomenon—(1) the deterioration of tetanolysin, and (2) the true "Danysz Effect." It would seem then to be impossible, from experiments with tetanolysin, to arrive at a general interpretation of the "Danysz Effect," applicable, for example, to diphtheria toxin, by the simple procedure adopted by Arrhenius in his calculations. The basis upon which the equivalence of the lysin and antilysin has been estimated is still open to many of the grave doubts advanced by Nernst (1904) and Craw (1905, I.) (1905, III.).

The purely arbitrary assumption is made that one "molecule" of tetanolysin combines with 1 "molecule" of antilysin to produce 2 "molecules" of compound; the formula used in the interpretation of the experimental results was, namely,

$$\frac{1}{T_0} \left\{ np \frac{1}{T} - \left( \frac{1}{T} - \frac{1}{T_0} \right) \right\} = K \left( \frac{1}{T} - \frac{1}{T_0} \right)^2,$$

where  $T_0$  represents the original or total amount of toxin and  $T$  the amount left free,  $n$  the number of c.c. of antitoxin,  $p$  a constant indicating the ratio between units of toxin and antitoxin and  $K$  a supposed equilibrium constant.

Now, as has been pointed out by Nernst (1904), the use of two constants  $p$  and  $K$  in this equation practically reduces it to an interpolation formula, and this seems to me to be confirmed by the fact that in the calculations made on other nearly related reactions in immunity Arrhenius and Madsen have been compelled to modify the power to which the right hand member of the equation is raised.

That the equivalents between toxin and antitoxin so deduced have no relation to those enunciated by Ehrlich is obvious, from the fact that a mixture of Arrhenius and Madsen's equivalents has a toxicity equal to 23.7% of that of the original toxin; such a mixture can only be described as "neutral" when all their assumptions are supposed to be correct. The definition of a "neutral mixture" is obviously purely arbitrary, and, in deference to the methods of Ehrlich, in my paper (1905, III.) on the toxin-antitoxin reaction, I defined a "neutral mixture" of megatheriolysin and antilysin as one which, after heating for three hours at 37°C., just failed to give a trace of haemolysis on heating for a further two hours under standard test conditions. All the mixtures which I investigated would from the point of view of Arrhenius contain excess of antitoxin. In such mixtures Madsen and Arrhenius find that the "Danysz Effect" increases when the first fraction of toxin is allowed to remain for longer periods, or "reaction times," in contact with the antitoxin, before the addition of the second fraction.

On this doubtful basis Arrhenius has calculated the Danysz Effect, and he makes further assumptions that seem to me even less tenable, as will be shown below. It was found that 0.72 c.c. of tetanus antilysin was "equivalent" to 4 c.c. of a certain brew of tetanolysin, and that an "equilibrium constant" could be deduced. The "Danysz Effect" was then determined by bringing 0.8 c.c. of the antilysin in contact with

4 c.c. of lysin but in two fractions, the first being 1 c.c. and the second 3 c.c. The first fractions in various series of experiments were allowed to stand for different lengths of time at 37° C., the second fractions were then added and the whole heated for 30 minutes at 37° C.

The toxicity of a mixture, made by adding to the antilysin 4 c.c. of lysin at once, was taken as unity and a comparison was made with the haemolytic values of the other mixtures. In the table column (1) gives the time during which the first fraction of lysin was heated with the antilysin, and column (2) the toxicity of the final mixtures. The upper half of the table (A) refers to one brew of lysin and the lower (B) to a second.

TABLE (A) AND (B).

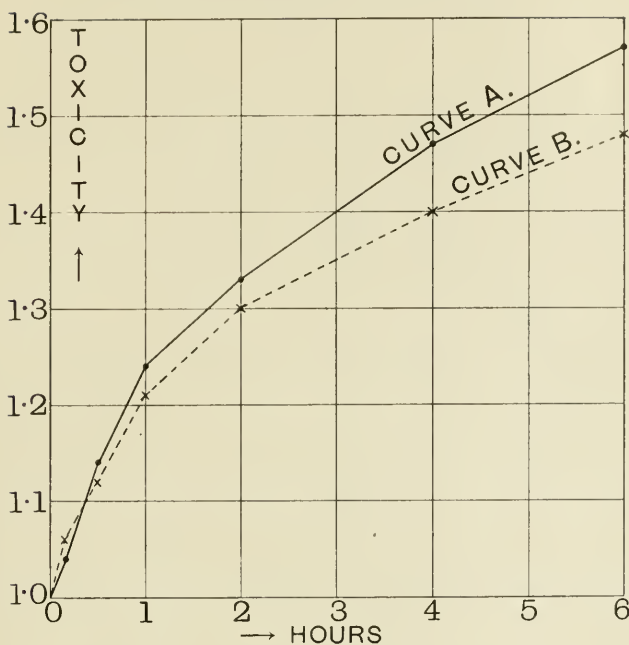
*The Danysz Effect.*

	Time	Toxicity	$E_{\infty} - E$	$K$	$E_{\infty}^* - E$	$K_1^*$	$K_2^*$
A.	0.0	1.00	60	—	90	—	—
	0.17	1.04	56	0.180	86	0.115	0.0030
	0.5	1.14	46	0.230	76	0.146	0.0041
	1	1.24	36	0.222	66	0.134	0.0040
	2	1.33	27	0.173	57	0.099	0.0032
	4	1.47	13	0.168	43	0.080	0.0030
	6	1.57	3	0.217	33	0.056	0.0032
	$\infty$	1.60	0	—	—	—	—
	$\infty^*$	1.90	—	—	0	—	—
B.	0.0	1.00	52	—	70	—	—
	0.17	1.06	46	0.318	64	0.228	0.0080
	0.5	1.12	40	0.228	58	0.163	0.0058
	1	1.21	31	0.225	49	0.155	0.0061
	2	1.30	22	0.187	40	0.122	0.0053
	4	1.40	12	0.159	30	0.092	0.0049
	6	1.48	4	0.186	22	0.084	0.0052
	$\infty$	1.52	0	—	—	—	—
	$\infty^*$	1.70	—	—	0	—	—

It will be observed that the effect of contact-time in the first fraction is very considerable and that with increment of time the phenomenon of Danysz increases, but with longer intervals, such as four hours and six hours, the rate of increase is less than with those below an hour. This will be better realised from the curves shown in the accompanying figure, which I have plotted from Madsen and Arrhenius's data.



## INFLUENCE OF CONTACT-TIME.



Curve (A) corresponds to the upper half (A) of the table and curve (B) to the lower half (B), toxicity being represented on the ordinates and time on the abscissae.

Arrhenius assumes that the "Danysz Effect" "appears to tend towards a limiting value," and that "one cannot easily imagine that the toxicity would increase with the time without limit."

These assumptions seem to me to be purely gratuitous, since from the curves which I have plotted the trend might, for lengthened periods, be as well parabolic as hyperbolic. Granting however that there may be a limiting value, is the magnitude chosen by Arrhenius in accord with the experiments quoted? It seems to me that this is not the case. In the table (A) the limiting value ( $\infty$ ) has been "estimated from the experiments" as 1.60, whereas from curve (A) in the figure it seems to me incredible that it would suddenly become parallel to the abscissa after say seven hours. Again in table (B) the limit ( $\infty$ ) is taken as 1.52, but from curve (B) no such value is warranted. It is true that these selected values agree best with Madsen and Arrhenius's theoretical views, expressed subsequently, viz., that the "Effect" obeys the law of

a monomolecular reaction, but the question is whether the data that they have furnished us with do not point to some quite other conclusion. It seems to me that this is so, for if a limit exist to curve (A) it would by graphical interpolation lie in the neighbourhood of 1.90, indicated in table (A) by  $\infty^*$ , and for curve (B) it would be nearly 1.70; using these values it becomes apparent on calculation by the method adopted by Arrhenius that the monomolecular formula does not hold.

In the third column of the table the differences between Arrhenius's limiting "Effect"  $E_\infty$  and the "Effect"  $E$  at any particular time are reproduced—the value being multiplied by 100; in the fourth column the values of  $K$  represent the constant obtained by manipulating the differences,  $E_\infty - E$ , as if a monomolecular reaction were being dealt with. The values of  $K$  do not appear to me as showing any remarkable constancy; further the first value in table (A), column (2) is too low and that of table (B) too high to render the curves smooth. If an intermediate value be chosen, *e.g.* 1.055 for table (A), a difference well within the experimental error, a much smoother curve is obtained and then it is found that the value of  $K$  shows a gradual diminution throughout five of the six members of series (A) and also of series (B). This in itself indicates that the monomolecular formula does not exactly represent the experiments. Now let us consider the effect of similar manipulations when we take the values of the limits,  $E_\infty^*$ , which I have provisionally interpolated. The fifth column indicates the new differences  $E_\infty^* - E$  and the sixth gives the values of  $K_1^*$  which should be constant if the monomolecular formula is applicable.

The uniform diminution of  $K_1^*$  and the magnitude of the decrease indicate that the formula does not apply even approximately. Moreover, when the above-mentioned intermediate value for the first "Effect" in table (A) is used, the magnitude of  $K_1^*$  diminishes throughout the entire series both of (A) and (B). In the equation  $\frac{dE}{dt} = K(E_\infty - E)^n$ , where  $t$  is the time and  $n$  a constant; supposing this equation to be applicable, I conclude that  $n$  is not equal to unity.

The question now becomes, is there any value of  $n$  which will give a constant value for  $K$ ? This seems to be the case, for, retaining the values of the "Effect" given in the second column and taking  $n$  equal to 2, a relatively high degree of constancy was obtained for  $K$  in both series (A) and (B). The results are shown in the seventh column  $K_2^*$  and indicate that the course of the "Danysz Effect" may be represented by the formula used for bimolecular reactions with close approximation.

If in table (B) the first "Effect"  $E$  be taken as 1.05 instead of 1.06, a value which gives a better fitting curve (B) and a difference below the experimental error, the constant  $K_2^*$  becomes 0.0065 instead of 0.0080 and the agreement with the bimolecular formula is therefore highly satisfactory. On the other hand, if in table (A) the above-mentioned value 1.05 be taken as the most probable for the first "Effect" the constant  $K_2^*$  becomes 0.0043, and thus there is a very slight falling off of  $K_2^*$  throughout the series (A), which indicates that the magnitude of  $n$  probably only differs from 2 by a small additional fraction and further it is certain that the value  $n=3$  is, by far, too great. It does not seem to me profitable to pursue the re-calculation of the meagre data placed at our disposal, and it is futile and perhaps misleading to give, as Arrhenius does, the toxicities which have been calculated by means of a constant derived from the experimental results. If a constant be obtained, that in itself is sufficient to prove the validity of the formula; and the subsequent calculation of toxicity and comparison with observed toxicity in tabular form are liable to give rise to a false impression in the minds of workers in Immunity, who have often but slight knowledge of the methods of estimation, and lead to the belief that the theoretical views underlying the arithmetical manipulations have been substantiated. Further, as the remainder of Arrhenius's calculations depends upon estimated limiting values,  $E_\infty$ , of the "Danysz Effect," which are probably open to objections of a similar nature to those advanced above, we must remain in doubt as to the actuality of the apparent correspondence between the experimental and calculated results until the original data are published.

In illustration of this I may cite Arrhenius's manipulation of the evidently valuable experimental work of Madsen and Walbum on the influence of excess of antitoxin on the "Danysz Effect."

Madsen finds that a constant quantity of toxin, viz. 4 c.c. added in two fractions of 1 c.c. and 3 c.c. to a quantity of antitoxin varying from 0.2 c.c. to 1.2 c.c. gives a "Danysz Effect" which is practically proportional to the amount of antitoxin present. Arrhenius assumes that this strict proportionality holds when the antitoxin is further diminished, and concludes that the "Effect" would disappear when the quantity of antitoxin used is less than 0.16 c.c. This view does not seem to me to harmonise with Arrhenius's own conceptions, for even with 0.16 c.c. of total antitoxin there should be a considerable proportion free which should be subject to the same laws of change ascribed by Arrhenius to excess of antitoxin. Further, when no antitoxin is added there can be

no "Effect" and when a great excess is present the "Effect" should be negligible, consequently with increasing quantities of antitoxin a gradual rise in the value of  $E_{\infty}$  is to be expected for very small quantities followed by an increase approximately proportional to the added antitoxin until a maximum value is reached, after which the "Effect" would gradually diminish. It does not, then, appear to me that these experiments give a method of determining the equivalents of toxin and antitoxin and consequently they do not form a "very strong support" of the view of Madsen and Arrhenius.

With regard to the application of Madsen and Walbum's experimental results to the theory of the toxin-antitoxin reaction, the increment in the "Danysz Effect" with increasing antitoxin throughout such a long range of concentrations seems to me to indicate that it cannot be due to a modified toxin, epitoxinoid, having the properties assumed by v. Dungern, for with greater quantities of antitoxin more should be left free to combine with the toxin and the "Effect" should diminish; this however may depend upon the range of antitoxin concentrations selected, as indicated above. To go to the root of the matter, Madsen and Arrhenius have advanced no theoretical justification of their treatment of the differences,  $E_{\infty} - E$ , as a monomolecular reaction, and no grounds why this relation between toxicities should be ascribed to the antitoxin. It has been seen that the formula used is merely an interpolation, without, as yet, any definite significance from the point of view of the mass law of Guldberg and Waage, and further, as such, it is probably incorrect. Moreover, the introduction of a new modification of "Antitoxin" which reacts more slowly with the toxin, but fixes it more firmly, and during which 1 "molecule" of toxin probably binds 2 "molecules" of antitoxin, renders the explanation of Madsen and Arrhenius as complicated as that of v. Dungern. This is the more to be regretted as Arrhenius and Madsen's views on Immunity have been confirmed in many respects and have the advantage of relative simplicity. It was then with some curiosity that I had recourse to the third view of the "Danysz Effect," viz. that of Bordet. Bordet (1903), Craw (1905, I.) and Bayliss (1906) have shown that a very similar effect is obtained in the staining of filter paper by anilin dyes, the paper being regarded as the antitoxin and the dye as the toxin. This "Danysz Effect" in staining is regarded as belonging to that class of phenomena called "Adsorption," the quantitative investigation and theoretical interpretation of which are at present subjects of numerous researches. It became apparent on the first few days' investigation that there



is a high probability that the relations existing between staining substances such as fuchsin, methylene blue, methyl green, erythrosin, etc., and absorbent matter such as filter paper, porcelain and ball clay are of an entirely similar nature to those described by Madsen and Arrhenius in their extensive work on "The Danysz Effect" in mixtures of toxin and antitoxin, viz. (1) increment of the time interval between the addition of the fractions of dye increases the amount of dye left free, and (2) the "Effect" is augmented by increment in the amount of absorbent material, throughout a certain range. The experimental work, which is at present in hand, on these matters will shortly be published.

These results strengthen the views advanced by Craw (1905, I.) (1905, III.) in support of the interpretation of immune reactions initiated by Bordet (1903) and Landsteiner (1903) and supported by Biltz (1904), Nernst (1904), Bayliss (1906), Freundlich (1906), and others. In my paper (1905, III.) it was shown that Arrhenius and Madsen were correct in assuming that free toxin and free antitoxin exist side by side in all mixtures of the two substances. Atoxic mixtures on filtration through gelatin became toxic, whereas the residual unfiltered fluid was antitoxic. This of course has nothing to do with the "reversibility" of the reaction as Arrhenius (1907) erroneously concludes in his *Immunochemie*, p. 18. The fact that the toxin-antitoxin combined together may be separated in part, but in part only, is shown however by other experiments in the same paper (1905, III.); this is the meaning I attached to the term "partially reversible." It will be observed that Madsen and Arrhenius (1907) have been compelled to assume this incomplete reversibility, as they now suppose that their "modified antitoxin" binds the toxin more firmly than the original antitoxin did.

Further as the toxin-antitoxin reaction especially at 20° C.—the temperature at which a considerable number of observations with tetanolyisin have been made—requires an appreciable time for the completion of the union, the mechanism of the "Danysz Effect" must also operate when the whole quantity of toxin is added to the antitoxin at once; consequently the original relatively simple and now complicated views of Arrhenius and Madsen are as inapplicable as those of v. Dungern and Sachs, for the values of the equivalents of toxin and antitoxin of both of these schools of Immunity must be seriously influenced in many cases by what has been termed "false equilibrium," but really means an equilibrium which is not of the type met with in



the neutralisation of acids by bases. Reverting then to the standpoint of adsorption it seems to be admitted, even by Arrhenius, that the antitoxins are colloids, but as regards the toxins it is still a matter of doubt to what, if any, extent they are colloidal.

Arrhenius (1907) erroneously attributes to me the assumption that the toxins are colloidal and act as fine suspensions. I found (1905, III.) however that antitoxin does not appreciably diffuse through gelatin. The results arrived at by Arrhenius and Madsen (1902), which they consider show a "marked diffusibility" and of which Arrhenius (1907) seems to believe I had no knowledge, but which I personally discussed with Madsen shortly after their publication, seem to me capable of another interpretation, viz. that by the superposition of an aqueous solution of antitoxin over a gelatin column the transmission effect may not be due to diffusion but to imbibition, as I had found for megatheriolysin (1905, III.).

In my experiments the toxin, antitoxin, or mixtures, were contained in a gelatin layer superimposed on a column of gelatin, and imbibition effects thereby eliminated.

Arrhenius (1907, p. 19) appears to be unfamiliar with the mechanism of the gelatin filter. This method (Craw, (1906)) gives an indication in a few minutes of the crystalloidal or colloidal nature of a solution—under certain conditions, a confirmation of C. J. Martin's (1896) view—whereas a similar differentiation by means of dialysis or diffusion would require days or even weeks. Arrhenius has failed to grasp the meaning of my remarks on suspensions (1905, III.). The "theoretical considerations" had the object of showing that the suspension view was untenable if the union of toxin and antitoxin were purely chemical. The conclusion arrived at was that the toxin-antitoxin reaction had the greatest number of points of analogy with adsorption phenomena. This view seems to me to be materially strengthened by the experimental work of Madsen and his pupils. The calculations of Arrhenius are in my opinion of doubtful value and afford inadequate support to the purely chemical interpretation of the reaction between toxin and antitoxin.

#### SUMMARY OF CONCLUSIONS.

1. It is inadmissible to study the "Danysz Effect" on tetanolysin owing to its rapid deterioration.
2. The so-called "equivalents" of toxin and antitoxin deduced by Arrhenius and Madsen are arbitrary.

3. No evidence has yet been advanced that the "Danysz Effect" has a limiting value when the time of contact of the first fraction of toxin with the antitoxin is prolonged.

4. If a limiting value of the "Danysz Effect" exist that calculated by Arrhenius is probably erroneous.

5. The monomolecular formula used by Arrhenius is merely an interpolation.

6. The "Danysz Effect" is much better represented by a bimolecular formula.

7. No confirmation of the "equivalents" of toxin and antitoxin has as yet been obtained from the "Effect."

8. Expediency appears to be the only justification for assuming that the "Effect" is due to either a modified antitoxin or to a modified toxin, viz. epitoxonoid.

9. All the phenomena of the "Effect" hitherto advanced have their counterpart in the staining of paper, porcelain, etc., by anilin dyes.

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## ON THE ESTIMATION OF FREE DIPHTHERIA TOXIN :

WITH REFERENCE TO THE RELATIONS EXISTING BETWEEN  
LETHAL DOSES, LETHAL TIMES AND LOSS IN WEIGHT OF  
THE GUINEA-PIG.

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THE unit lethal dose of a fluid containing free diphtheria toxin may be defined, by Ehrlich's method of standardisation, as the smallest quantity of the fluid which, diluted to 4 c.c. with saline or water and injected subcutaneously into a healthy guinea-pig of 250 to 280 grams weight, causes death in 4 to 5 days. The standardisation of a toxin is usually made in two ways—(1) various dilutions of the toxin are injected and the unit or minimal lethal dose, M.L.D., obtained; (2) various dilutions of the toxin are mixed with an empirically fixed quantity of antitoxin—the Ehrlich immunisation unit, I.E.<sup>1</sup>—and are injected into a series of guinea-pigs. By this test the amount of toxin necessary to neutralise the unit of antitoxin and still leave a lethal dose in excess, usually denoted as the L+ value of the toxin, is determined. When the quantity of toxin is, in both cases, greater than the unit lethal dose the time required for death or lethal time is shortened, and with smaller quantities of toxin death is delayed beyond the standard time. Further, lethal doses usually produce a daily diminution in the weight of the test animal until death ensues, and sublethal doses give rise to a temporary decrease in weight.

<sup>1</sup> Immunisierungseinheit.

In a recent investigation of the neutralisation of diphtheria toxin by antitoxin, Arrhenius and Madsen (1904) have deduced a quantitative relation between lethal dose and lethal time, and likewise an empirical connection between dose of toxin and loss of weight in the test animals. If the observations of Arrhenius and Madsen be found to agree with those on other brews of diphtheria toxin obviously standardisations would be rendered more accurate or a saving in animal life could be effected. In their further experiments, designed to test the validity of their views on the toxin-antitoxin reaction, Arrhenius and Madsen find that their observations, interpreted according to the above-mentioned relations, are in close agreement with the theoretically derived values for free diphtheria toxin. Nernst (1904) has pointed out that the equation used by Arrhenius and Madsen is merely an interpolation formula, and the close agreement between calculated and observed results does not prove the validity of their hypothesis. Their observations, however, seem to show that diphtheria toxin is gradually and continuously neutralised by increasing amounts of antitoxin, a result which is in opposition to the experimental investigations and views of Ehrlich (1898), (1903), according to whom the neutralisation takes place in steps owing to the successive combination of several distinct toxic and atoxic substances with different combining affinities present in the toxin. From Arrhenius and Madsen's point of view the step-like neutralisation found by Ehrlich and his pupils is to be interpreted as, in part, due to experimental errors, and the smoothness of the neutralisation curves which they, themselves, have obtained is, in part, due to the more profitable interpretation of their experimental results gained by the application of empirical relations between lethal doses, lethal times, and loss of weight.

It seemed, therefore, to the authors to be important to control the relations deduced by Arrhenius and Madsen, both from the standpoint of toxin and antitoxin standardisation, and the physico-chemical investigation of the toxin-antitoxin reaction by experiments *in vivo*. Fortunately an extensive record of data had been collected by one of us at the Lister Institute of Preventive Medicine during his standardisation of antitoxic sera, within the period 1900 to 1905, and these observations have been subjected to detailed analysis by the other. In this communication the chief features of a preliminary examination of some of these observations are presented, pending an investigation by more refined statistical methods, such as those of Prof. Karl Pearson. One of us, viz. G. Dean, is responsible for the experimental data alone, and the other, J. Craw,

for the calculations and conclusions, carried out during his tenure of scholarships from the "Lister Institute," the "British Medical Association" and the "Grocers' Company."

*Lethal Doses of Toxin and Time of Death.*

In Table I the relations found by Arrhenius and Madsen to hold between lethal doses and lethal times in days, columns 1 and 2, are reproduced. In this connection that quantity of toxin which killed with

TABLE I.

*Toxin-Time Relations. (Arrhenius and Madsen.)*

Time	Dose	<i>R</i>	Gm.	Dose
1	1·6	40	120	1·30
1·5	1·4	—	100	1·20
2	1·25	52	80	1·10
2·5	1·15	—	65	1·00
3	1·05	70	60	0·92
3·3	1·0	—	55	0·85
3·5	0·97	—	50	0·78
4	0·91	87	45	0·70
4·5	0·85	—	40	0·61
5	0·80	100	35	0·55
6	0·71	98	30	0·50
7	0·64	—	20	0·45
8	0·59	78	10	0·40
9	0·55	—	0	0·35
10	0·51	51	<0	0·35
12	0·45	—	—	—
14	0·40	11	—	—
>14	0·40	—	—	—

certainty in 3·3 days was taken as unit toxin or minimum lethal dose, M.L.D., but it is of course easy to calculate proportional series of doses when another time limit is selected for the M.L.D., *e.g.* 3, 4 or 5 days; thus in Table II we give the lethal doses obtained from Arrhenius and Madsen's relation for the second to the sixth day on the basis of a 3-day unit or M.L.D., series "L. dose A. and M." The dotted curve with crosses in Fig. 1 gives a graphical representation of this relation, the lethal doses being shown on ordinates, and the corresponding lethal times on the abscissae. Further, Arrhenius and Madsen assume that if a guinea-pig recovers, its maximum loss of weight will, in general, occur on the fifth day after inoculation and that the loss in weight on any particular day up to the fourteenth will bear a definite ratio to the maximum.



Thus, if the maximum loss on the fifth day be taken as 100 the decrement in weight on the first day would have the relative value 40. This series of ratios is reproduced in Table I, column 3 (*R*), and it is possible, by means of it, to calculate from the loss of weight on the first or the second, third, etc. day, what the maximum loss of weight would be on the fifth day, as the absolute loss on any particular day has merely to be multiplied by a definite known ratio. In this manner the loss in weight on each day, both of pigs which survive and of those which die, gives a calculated value for the maximum for which an average can then be obtained. Arrhenius and Madsen consider that a definite relation exists between the maxima so obtained and the dose of toxin injected, and this is reproduced in Table I, columns 4 and 5. The series of weight ratios is represented diagrammatically in Fig. 2, the abscissae giving the time in days and the ordinates the ratio scale 0 to 100.

TABLE II.

*Toxin-Time Relations.*

Lethal time in days	2	3	4	5	6
Lethal dose	1.8	1.1	0.8	0.7	0.65
Range of dose	5—1.4	2—1.0	1.2—0.7	0.9—0.5	0.8—0.45
L. dose (A. & M.)	1.2	1.0	0.87	0.76	0.67

The uncertainty associated with the estimation of free diphtheria toxin, owing to variation in the susceptibility of individual guinea-pigs, appears to us to bring these determinations into the class of phenomena which must be treated by more general methods of probability. The deviation from the most probable result decreases as the number of pigs tested increases; thus the problem is to find the likelihood of a result derived from a limited number of tests agreeing approximately with an expected value, provided we proceed on the basis of an hypothesis such as that of Arrhenius and Madsen. If, on the other hand, we proceed according to the method of Ehrlich the probabilities are unknown and we have the inverse problem, viz., to elucidate the M.L.D. and L+ values and their theoretical significance from the experimental data. In the present preliminary communication we have adopted the latter method to control the relations between lethal time, dose of toxin and loss of weight. It may, in parenthesis, be remarked that Ehrlich's rectangular method of representation of the neutralisation of toxin by antitoxin would, if carried out systematically, give a better idea of the

exact value of the experimental data, but that Arrhenius and Madsen's curves give a better idea of the course of the reaction.

In order to control this relation the data referring to 200 different brews of diphtheria toxin were tabulated, and average values were found for the relation between lethal doses and lethal times. Owing to the large number of estimations the arithmetic and geometric means did not differ appreciably.

The range of dose causing death on any particular day is considerable: for the first day it is very great and no upper limit can be given, for the second to the sixth days the ranges are given in Table II, for durations exceeding seven days no relation whatever could be found. In all cases the values given are the arithmetic means. It may be observed that there are more terms<sup>1</sup> obtainable from any single series of estimations of diphtheria toxin than there are constants, and consequently all the terms must be used in deducing a relation between lethal times and lethal doses. Such a relation has been found for the above-mentioned 200 toxins and is given in Table II, Series 1 and 2, the graphical representation of which is to be seen in the continuous curve of Fig. 1. This graduation curve does not reproduce any of the observations exactly, but it runs evenly through the roughness of the observations and so indicates their general trend. These average values are also probably not a true representation of the experimental data, but only an approximation; it seems to us not improbable that the "mode," *i.e.* the lethal dose which on a particular day most often causes death, is probably a better guide to the toxin-time relation than the "mean," for owing to the spread or scatter of the observations being, in any selected day, unsymmetrical about the mean and congregating during the first, second and thirds days in the neighbourhood of the longer periods, the frequency curves exhibit a pronounced skewness, a matter which will be dealt with in another paper. This may be gathered from Fig. 1, in which the black circles on the continuous curve represent the means, whereas the straight lines corresponding to each circle represent the ranges—the deviation from symmetry is apparently not a negligible factor.

Omitting for the present the toxin-time relation for the first day, it will be seen, from Table II and Fig. 1, that the lethal dose 1.2 obtained by Arrhenius and Madsen for the second day differs considerably from our values, *viz.* 1.8, but that on the third day the values correspond

<sup>1</sup> *i.e.* lethal values for the series of days considered.

closely, and on the fourth, fifth and sixth days are practically identical. The relation we have obtained seems capable of simpler expression than that of Arrhenius and Madsen, for we have the approximate relation that

$$\text{Lethal Dose} \times \text{Lethal Time} = \text{Constant.}$$

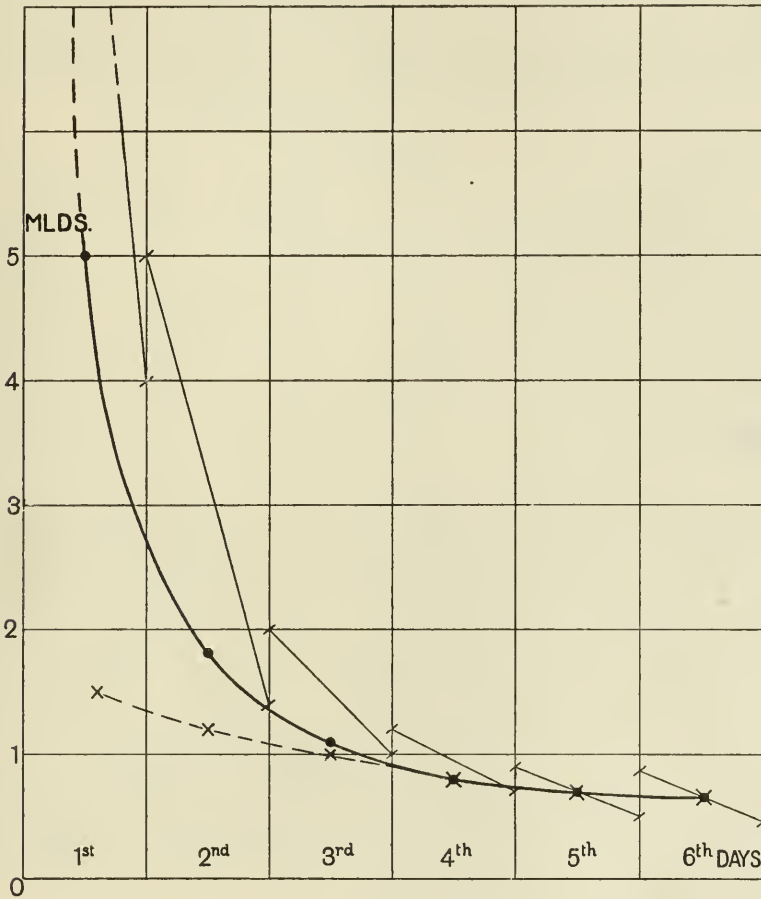


Fig. 1.

Thus the doses killing from the second to the sixth day give the products 3.6, 3.3, 3.2, 3.5 and 3.9. The corresponding products from Arrhenius and Madsen's relation are 2.4, 3, 3.48, 3.80, 3.92. The simple relation given, although probably not exactly of the best fitting type, which seems to be  $\text{Lethal Time} \times \sqrt[n]{\text{Lethal Dose}} = \text{Constant}$ , where  $n$  is a second constant, is likely to prove serviceable as a rough guide, giving

518      *The Estimation of Free Diphtheria Toxin*

results within the experimental error in the customary series of five guinea-pigs used in the standardisation of diphtheria toxins. It is certainly a useful mnemonic, for it states that the time of death is nearly inversely proportional to the dose of toxin. It seems to us to be doubtful whether any closer approximation is attainable under ordinary circumstances as regards the number of guinea-pigs likely to be used in standardisation. This is clearly shown by Table III for Toxin, No. 116, where the number of deaths are given and the days upon which death took place when various amounts of toxin were administered.

TABLE III.

*Diphtheria Toxin No. 116: filtered 28/1/01. Tested 1904.*

Toxin in c.c.	No. of pigs	Time of death † in days								No. of survivals
		1	2	3	4	5	6	7	†days	
0·01	3	—	—	††	†	—	—	—	—	—
0·0075	1	—	†	—	—	—	—	—	—	—
0·005	4	—	†	††	—	†	—	—	—	—
0·004	3	—	—	†††	—	—	—	—	—	—
0·0033	5	—	—	—	†††	—	—	†	†30	—
0·0029	3	—	—	—	—	—	—	—	†10	2
0·0025	3	—	—	—	—	—	†	—	†24	1

It is noteworthy that a definite lethal amount of toxin cannot be relied upon to kill on any particular day, but the probability is that it will kill on one of two days immediately succeeding each other. This table seems to us to be typical of the usual experience met with in the determination of minimal lethal doses, for even in this case, where 22 guinea-pigs were used as test animals, the lethal dose 0·005 c.c. could not be relied upon to kill with absolute certainty on the third day, nor did 0·0033 c.c. give a lethal effect always on the fourth.

Further, the examination of the above-mentioned 200 brews of diphtheria toxin demonstrates conclusively that the number of animals dying on the seventh day to about the twenty-first day is so small and the irregularities so great as to render any calculation of a lethal dose for this period of no practical value.

*Lethal Effect of Toxin-Antitoxin Mixtures.*

As the L + dose of toxin denotes that quantity of toxic fluid which neutralises the unit of antitoxin, i.e., and still gives a minimal lethal dose in excess, it is evident that when the time limit for the L + dose is varied from the second to the sixth day the relative values will approxi-

mate more closely than we found to be the case for lethal doses of toxic fluid alone. This can be seen from Tables IV and V. In Table IV the range of L + dose killing on any day after inoculation is given from the year 1901 to 1905 for the best investigated toxin, viz. No. 116. The average values are shown in the last series, and taking the L + dose for

TABLE IV<sup>1</sup>.

*Toxin No. 116 in dilution 1:5, L + in c.c.*

Time of death in days					Date
2	3	4	5	6	
·6	·61	·63	·61	·60	April—Sept. 1901
·65	·75	·70	·62	—	
(4)	(8)	(3)	(3)	(1)	
·72	·70	·64	·74	·66	Oct.—Oct. 1901-2
·78	·76	·70	—	·68	
(13)	(14)	(2)	(1)	(2)	
·68	·68	·68	·70	·68	Oct.—Oct. 1902-3
·78	·74	·74	—	—	
(14)	(8)	(5)	(1)	(1)	
·70	·68	·68	·66	·68	Oct.—Oct. 1903-4
·74	·76	·72	·74	·70	
(3)	(10)	(4)	(3)	(3)	
·70	·68	—	·68	—	Oct.—Feb. 1904-5
·74	·74	—	—	—	
(3)	(8)	—	—	—	
·73	·72	·70	·70	·68	1901-5

TABLE V.

Lethal time. Days	L + doses corresponding		
	No. 116	No. 58	Mean
2	1·015	1·02	1·017
3	1·00	1·00	1·000
4	0·97	1·00	0·985
5	0·97	0·97	0·970
6	0·95	0·97	0·960

the third day as unity the relative values for death on the other days are indicated in Table V. From Table V it may be seen that the range of dose for any particular day is considerable, but the "scatter" of the data is such that the mean values have an experimental error of  $\pm 2\%$  at the outside.

<sup>1</sup> Bracketed figures indicate number of test animals.



A very similar relation was obtained for another toxin, No. 58, as is indicated in Table V. Taking the mean of these observations, we find that the L + doses and corresponding lethal times show a straight line relationship.

It follows also from Table IV that the neutralising power of the toxin No. 116 did not change appreciably during the period October, 1901, to February, 1905, although during this period the minimum lethal dose had increased by 100%, viz. 0·002 c.c. to 0·004 c.c. This is confirmatory of Ehrlich's view that on standing the toxic property of diphtheria toxin diminishes, whereas its neutralising power is, within the experimental error, unchanged. From April, 1904, onwards the toxicity of No. 116 appears to have remained constant, and the curve representing the decrement with time of standing is probably of hyperbolic form and similar to those found by Madsen for the disappearance of antitoxin from the animal system, the deterioration of tetanolysin, etc. A corresponding diminution in toxicity and constancy of neutralising power was observed for toxins Nos. 58, 27, 136 and 198.

The influence of the variation in the susceptibility of the test animals is also markedly shown on injecting mixtures of toxin-antitoxin. Table VI gives a summary of the results obtained when

TABLE VI.

*Lethal Effect of Toxin-Antitoxin Mixtures.*

Toxin No. 116 in dilution 1:5+1 I.E.

Toxin c.c.	Pigs No.	Days						Survivors	
		1	2	3	4	5	6	No.	%
1·2 —0·8	10	6	4	0	0	0	0	0	0
0·8 —0·75	15	1	9	4	0	0	0	1	6·6
0·75—0·7	33	0	10	15	2	1	0	5	15
0·7 —0·65	20	0	2	7	3	0	2	6	30
0·65—0·6	32	0	5	4	2	3	1	17	53
0·6 —0·5	16	0	1	0	0	0	1	14	87
0·5 —0·4	10	0	0	0	0	0	0	10	100
0·4 —0·3	8	0	0	0	0	0	0	8	100
0·3 —0·2	5	0	0	0	0	0	1	4	80

different amounts of toxin were used, the fluid containing unit antitoxin, *i.e.* 1 I.E., in each case. For example, with doses between 0·7 and 0·75 c.c. of a 1 in 5 dilution of toxin No. 116, out of 33 guinea-pigs, 10 died on the second day, 15 on the third, 2 on the fourth, 1 on the fifth, 0 on the first and sixth, while 5 pigs, or 15% of the whole number, survived the sixth day. It seems to be clear from this table, which we

regard as very typical of the phenomena met with in investigating L+ doses, that the determination of the dose corresponding to any particular lethal time must be the result of the interpolation of a large number of experiments, and that any relation found to exist between lethal dose and lethal time can form, at the best, only a rough guide in the estimation of free toxin in mixtures of toxin and antitoxin.

*Loss of Weight and Lethal Time.*

An endeavour was made to control the relations deduced by Arrhenius and Madsen between the variation in weight of the test animals and the time elapsing after inoculation, all the series of Table VI for toxin No. 116 being examined and mean values obtained for loss of weight on any particular day from the first to the fourteenth. The first and most striking result was that, although guinea-pigs which recovered showed at some period a maximum loss of weight, this maximum did not occur for all doses on the fifth day. Further, the shapes of the curves connecting lethal time and loss of weight were not similar to that which represents Arrhenius and Madsen's ratios (see Fig. 2). Quite other relations seem to exist; in the first place, non-lethal doses do not depress the weight of the test animals as a rule on the first day, but the weight continues to rise, and, with the smaller doses, at quite a normal rate, as will be seen from a subsequent paper. With larger doses the weight at the end of the first day may be constant, slightly increased or slightly depressed. Only with large lethal doses, in general, is a marked depression obtainable. Secondly, the injection of sub-lethal doses, in which the antitoxin is in great excess, leads in many cases to a marked temporary depression on the first day. This we attribute to "shock," for it has been observed that on the subcutaneous injection of perfectly innocuous fluids, such as normal saline, distilled water, distillates of broth, etc., a similar decrement of weight took place during the 24 hours succeeding inoculation. The mean values of a large number of tests show, however, that the relation first mentioned holds in general, viz., that after the injection of all mixtures of diphtheria toxin and antitoxin the animal continues to increase in weight, but that with increasing amounts of free toxin this increment in weight is more rapidly followed by a decrement, and with non-lethal doses the maximum weight of the pig is found at the end of 24 hours. It appears to us that these maximal weights would, if they were ascertained either by direct determination or by interpolation from a few observations, form

more reasonable origins for the weight-time curves corresponding to different doses. As an approximation, especially in view of the unreliability of the results obtained during the first 24 hours, we decided to take the weight at the end of the first day as the origin of each curve, or, in other words, as the initial weight of the test animal. On pursuing this course several relations, formerly obscured, became immediately apparent, and these may be gathered from Fig. 2, curves 1 to 6. For pigs which recovered no maximum loss in weight occurred for doses of 0.4 c.c. to 0.3 c.c. of toxin No. 116 corresponding to the eighth series of Table VI, as shown in Fig. 2, curve 6. Series 7, Table VI, gave curve 5

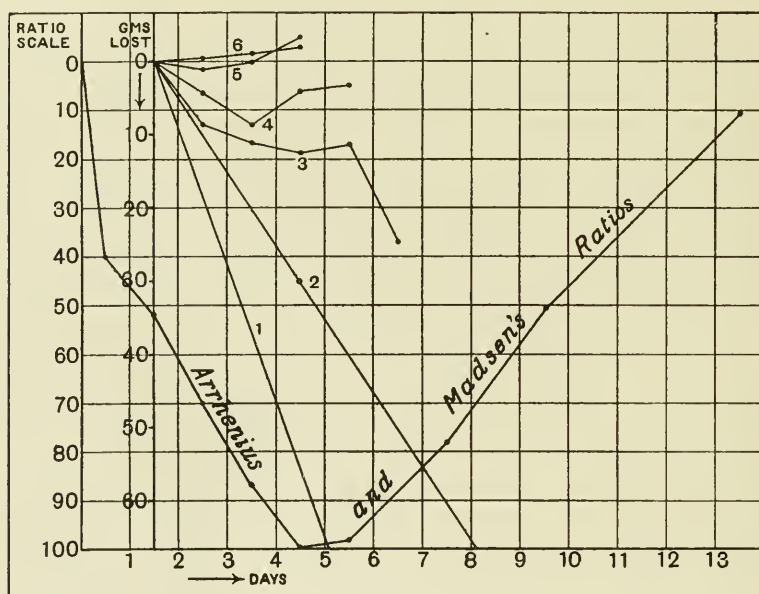


Fig. 2.

in the same figure, and indicates that with 0.5 c.c. to 0.4 c.c. of the diluted toxin the maximum loss of weight took place on the third day. Curve 4, belonging to series 6, Table VI, gives a maximum loss on the fourth day for 0.6 c.c. to 0.5 c.c. of the toxic fluid. For series 5 no definite maximum loss could be obtained, and, indeed, the form of curve 3 seems to indicate that this is a transition relation between those holding for pigs which recover and others applicable to pigs which die within the first few days. The general downward trend of the curve is probably due to the fact that only 53% of the test animals survived the sixth day

on the injection of 0.6 c.c. to 0.65 c.c. of toxic fluid. The remaining curves, 2 and 1, show in general the time-weight relations existing for amounts of toxin which can be more accurately described as lethal doses or M.L.D.S. and L + doses when antitoxin was present, and represent in the latter case the effects of amounts of toxin No. 116 varying from 0.65 c.c. to 0.8 c.c. of the dilution stated in Table VI. In this lethal zone, enclosed by the two curves now under consideration, the injection of 0.65 c.c. gives rise to curve 2 and increasing quantities of toxin give curves which incline more steeply to the abscissa, until finally with 0.8 c.c. the position occupied by curve 1 is reached.

With doses greater than 0.8 c.c. up to 1.2 c.c. the inclination does not appreciably exceed that of curve 1, and as the values obtained from these doses refer chiefly to pigs which died within 24 hours they have been omitted. The lethal rays thus obtained for the time-weight relations of doses between 0.65 c.c. and 0.8 c.c. are, within the experimental error, straight line relations, and it will be seen from a subsequent paper that they practically correspond to the "starvation curve" of the guinea-pig under normal conditions.

#### *Summary of Conclusions.*

1. The relation between lethal doses of diphtheria toxin and the times in which they kill guinea-pigs, or lethal times, is approximately such that the lethal dose multiplied by the corresponding lethal time gives a constant value—a hyperbolic relation.

2. Deductions with regard to lethal doses from deaths occurring on the first day or on any day after the sixth give such widely divergent results that, in our opinion, they are at present of negligible value.

3. Under the ordinary circumstances of standardisation of diphtheria toxin and antitoxin the relation given in conclusion No. 1 is as close an approximation as any alternative likely to prove of practical utility.

4. The graphic representation of L + doses against lethal times gives a straight line relationship.

5. Five of the best investigated toxins confirmed Ehrlich's views in so far as that with toxicities diminishing by 50% their neutralising powers remained practically unimpaired.

6. The individual sensitivities of guinea-pigs to free diphtheria toxin render any general relation between lethal times and doses of little value when a small number, *e.g.* 5, of test animals is inoculated.

7. Even with a dose of toxin which would, in any ordinary series of tests, be regarded as an L + dose, 5 out of 33 guinea-pigs survived the sixth day or 15%<sup>1</sup>, and the M.L.D. of a well-investigated toxin showed similar variations; consequently both the L + and M.L.D. values of a toxin are not those amounts which kill with certainty in a fixed time, but those which will cause death in that time with the greatest probability.

8. After inoculation, guinea-pigs unless suffering from the consequent "shock," continue to increase in weight throughout the first day, even with doses that may subsequently prove lethal, and with highly toxic doses the variation in weight during this time is of little significance.

9. The greater the amount of free toxin injected, the more rapid is the increment in weight followed by a decrement.

10. The weight of a test animal at the end of 24 hours after inoculation forms a better normal or origin with which to compare the subsequent time-weight relations than the initial weight before injection.

11. On the basis of Conclusion 10 the general connection is found that with increasing amounts of free toxin the position of the maximum loss of weight is gradually transferred from the second to the fifth day for guinea-pigs which survive over six days.

12. The time-weight ratios for almost certainly lethal doses give straight line connections, corresponding probably to starvation curves of the guinea-pig.

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<sup>1</sup> In the standardisation of diphtheria antitoxin at the Lister Institute, Elstree, 0.72 c.c. of toxin No. 116 was considered to represent the L + dose for the fourth or fifth day instead of the third, which, according to the above calculations, would harmonise best with the mean of all the observations. The fluids so standardised had then at least the value ascribed to them and were probably higher in content of antitoxin.



# THE TOXICOLOGY OF NICKEL CARBONYL.

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(Four Figures.)

## PART I.

### *Introduction.*

NICKEL carbonyl was discovered by Mond, Langer, and Quincke (1890). On passing a current of carbon monoxide over finely divided (pyrophoric) metallic nickel, they discovered that a gaseous compound of nickel and carbon monoxide was formed, having the composition of  $\text{Ni}(\text{CO})_4$ .

When the gas is heated to  $150^\circ \text{C}$ . it is decomposed into its constituents, and metallic nickel is deposited. The volume of liberated carbon monoxide after dissociation is four times that of the undissociated nickel carbonyl vapour. The gas can be condensed to a mobile liquid, if cooled in a refrigerating mixture. Fluid nickel carbonyl boils at  $43^\circ \text{C}$ . and solidifies into needle-shaped crystals at  $-25^\circ \text{C}$ . The vapour density at  $50^\circ \text{C}$ . is 86.9.

Further investigations on this compound were conducted by Mond and Nasini (1891), Berthelot (1901), Mittasch (1902) and Dewar and Jones (1903).

Nickel carbonyl is a clear, pale straw-coloured liquid, volatilising at room temperature. It has a peculiar soot-like smell, which can be detected in extremely small quantities (about 1 volume in 2,000,000), while the Bunsen flame becomes luminous when nickel carbonyl is present in air to the extent of 1 volume in 400,000. The liquid is soluble in alcohol, benzene and chloroform. It is not acted upon by alkalis nor by weak acids. Strong hydrochloric acid does not decompose it, but nitric acid and aqua regia do so easily.

Cobalt does not form a carbonyl, but iron does. Liquid iron carbonyl ( $\text{Fe}(\text{CO})_5$ ), when acted on by light, gives off one molecule of carbon monoxide, and a solid diferro-nonacarbonyl is formed, having the composition  $\text{Fe}_2(\text{CO})_9$ . Dewar and Jones (1906) also describe a tetracarbonyl of iron, which occurs in the form of short, dark green, lustrous prisms. It is almost insoluble and is not volatile. The pentacarbonyl is volatile at ordinary temperatures, but not to such an extent as the tetracarbonyl of nickel. It is made by passing carbon monoxide over reduced pyrophoric iron.

The discovery of nickel carbonyl was put to practical use by L. Mond for the manufacture of pure nickel. At first, it was not thought that the compound was more dangerous than the amount of carbon monoxide contained would suggest, and it appears that very little precaution was taken by the early investigators to avoid inhaling the vapour; fortunately without evil results.

However, when the substance was produced on a large scale, the accidental escape of the gas led to some unfortunate accidents, some four of which proved fatal.

The suggestion to investigate the toxicology of nickel carbonyl I owe to Dr Mond, who has placed a supply of nickel carbonyl and iron carbonyl at my disposal, and who has done much to render the work less difficult. Much assistance has been derived from the advice of his chemists, Drs Shields and Hirtz and also from Dr Langer, the Managing Director of the Mond Nickel Company's Works at Clydach, to all of whom I wish to express my thanks. Through the courtesy of Dr Mond I have been able to observe some mild cases of poisoning in man. It is therefore a great pleasure to me to express my thanks to Dr Mond in this place.

I wish also to express my grateful indebtedness to Dr Charles Martin, the Director of the Lister Institute of Preventive Medicine, for the very valuable assistance which he has given me, both in suggesting methods to overcome difficulties and also in criticising results; and to Dr Harden, whose advice on chemical matters has been a great help to me.

The symptoms of nickel carbonyl poisoning in man were as follows: immediately after having been exposed to air containing plant-gas, there was giddiness, and at times dyspnoea and vomiting. These symptoms passed off rapidly, as soon as the patients were brought into the fresh air. After from 12 to 36 hours, the dyspnoea returned, cyanosis appeared and the temperature began to be raised. Cough with more or less blood-stained sputum occurred on the second day or later.

The pulse rate became increased, but not in proportion to the respiratory rate. The heart remained normal. Delirium of varying types frequently occurred, and a variety of other signs of disturbance of the central nervous system was noted. Death took place in the fatal cases between the 4th and 11th days. The chief changes found post mortem were haemorrhages in the lungs, oedema of the lungs, haemorrhages in the white matter of the brain (in one case this was very extensive), while some doubt exists as to whether any blood changes were present.

At present it is not possible to state what the lethal dose for man may be.

In animals poisoned with nickel carbonyl vapour, analogous symptoms and post mortem changes have been observed. It is proposed to deal with these in detail in a subsequent communication. The post mortem changes in animals (rabbits, cats and dogs) consist of haemorrhages in the upper air passages, haemorrhages, oedema and compensatory emphysema of the lungs, haemorrhages in the adrenals and in the central nervous system. Haemorrhages occur less commonly in the kidneys, still less commonly in the spleen and very rarely in the liver.

The inhalation of air containing 0.018 volume % of nickel carbonyl vapour for 65 minutes is just sufficient to kill rabbits; 0.04 volume % has to be inhaled for 90 minutes by cats to produce death, while dogs die if exposed to 0.036 volume % for 90 minutes. No symptoms appear for the first 12 to 24 hours after the inhalation.

A few observers have studied the poisonous effects of nickel carbonyl. Henriot and Richet (1891) came to the conclusion that nickel carbonyl is taken up by the blood and is slowly split up. They found carbon monoxide haemoglobin. Langlois (1891) believes that nickel carbonyl replaces the oxygen in the blood. Vahlen (1891) discusses the conclusions of these authors and comes to the conclusion that death is due to nickel carbonyl as such and not to carbon monoxide. McKendrick and Snodgrass (1891) ascribe the toxic action to nickel, on the basis of a small number of experiments, chiefly dealing with enormously excessive doses. A number of earlier workers have dealt with the toxicology of nickel salts, among whom, Anderson Stuart (1884), Ross (1887), Riche (1888), Laborde and Riche (1888) and Rohde (1889) may be mentioned.

*The Toxic Agent in Nickel Carbonyl poisoning.*

In considering what the toxic agent in nickel carbonyl poisoning may be, one has to deal with three possibilities:

1. That the carbon monoxide of the compound is wholly or partly responsible for the symptoms;
2. that nickel carbonyl is absorbed as such and acts toxically;
3. that the nickel of the compound produces the symptoms.

A modification of these may be considered, viz. that nickel carbonyl is absorbed as such and that the nickel being set free later, acts toxically in the tissues.

1. *Consideration of what part in the Toxicology may be played by Carbon Monoxide.*

When nickel carbonyl is dissociated, the carbon monoxide liberated occupies four times the volume of the original vapour. Shields has pointed out that if nickel carbonyl is shaken up with blood, the haemoglobin acquires the colour characteristics of carbon monoxide haemoglobin. In my poisoning experiments, when the minimum doses of vapour were used, the blood contained only a small amount of carbon monoxide, which seldom exceeded 5 %.

In order to show that carbon monoxide is not the toxic agent, the following facts can be brought forward:

1. In fatal poisoning from minimal doses the actual quantity employed is insufficient when combined with the haemoglobin to produce more than 5 % of saturation with carbon monoxide, and this amount is harmless.

2. The blood of an animal saturated to the extent of 32 % with carbon monoxide loses carbon monoxide during subsequent poisoning by nickel carbonyl.

3. Iron carbonyl is less toxic than nickel carbonyl, although it contains more carbon monoxide.

Ad. 1. It has been found in a large number of experiments, that the inhalation of 0.018 volume % of nickel carbonyl vapour in air for 65 minutes is sufficient to kill rabbits; 0.04 volume % for 90 minutes is sufficient to kill cats, and 0.036 volume % for 90 minutes to kill dogs. It cannot be supposed that 0.072 volume %, 0.16 volume % and 0.144 volume % of carbon monoxide in air when inhaled by rabbits, cats and dogs for 65, 90 and 90 minutes respectively would produce

fatal results. These figures represent the amount of carbon monoxide liberated, if all the nickel carbonyl were dissociated. According to Gruber (1883) 0.07 to 0.08 volume % of carbon monoxide even when inspired for days does not kill rabbits. Gréhant (quoted by Sachs, 1900) found that dogs die when exposed to 0.4 to 0.5 volume % for from  $\frac{3}{4}$  to 1  $\frac{3}{4}$  hours.

During fatal poisoning in rabbits, samples of blood were taken and examined spectroscopically and by Haldane's carmine method (1895) for carbon monoxide haemoglobin. If carbon monoxide had been the toxic agent, one would have expected to find at least 50% of the haemoglobin saturated with carbon monoxide at the end of the inhalation period.

Ad. 2. Rabbits were exposed to carbon monoxide gas until the haemoglobin of the blood showed from 25 to 32% saturation. 30% of the haemoglobin of the first rabbit was saturated with carbon monoxide; the rabbit was then allowed to breathe air containing 0.0188 volume % of nickel carbonyl for 65 minutes, and died without exhibiting any additional symptoms. 25% of the haemoglobin of the second rabbit was saturated with carbon monoxide, and this rabbit was exposed to the same dose of nickel carbonyl. It too lived as long as the control and did not show any additional symptoms. 28.5% of the haemoglobin of the third rabbit was saturated with carbon monoxide and then the rabbit was exposed to 0.019 volume % of nickel carbonyl for 65 minutes. At the end of the inhalation the carbon monoxide haemoglobin had decreased to 25.7% of the total. Two other animals after inhaling sufficient carbon monoxide to saturate 25 and 32% of the haemoglobin of their blood respectively, recovered after severe illness on being poisoned by a dose of nickel carbonyl which killed 83.1% of rabbits subjected to it.

The toxic effect of nickel carbonyl is therefore not increased by a preliminary inhalation of carbon monoxide, provided that not more than 32% of the haemoglobin is saturated. The amount of carbon monoxide combined with the haemoglobin may actually diminish during the inhalation of nickel carbonyl.

Ad. 3. Nickel carbonyl contains less carbon monoxide than iron carbonyl. As has been stated, 0.018 volume % inhaled for 65 minutes of nickel carbonyl vapour in air is sufficient to kill rabbits. 0.025 volume % of iron carbonyl vapour is required to be inhaled for one hour to produce the same effects.

It will be shown subsequently that the course of the symptoms and



the death in nickel carbonyl poisoning is not like that of carbon monoxide poisoning.

*Conclusion.*

The foregoing facts prove that the toxic effect of nickel carbonyl is not produced by its carbon monoxide content. They also show that when the quantity of the nickel carbonyl vapour inhaled is only just sufficient to kill, the total available amount of carbon monoxide is insufficient to influence the course of poisoning.

*2. Consideration of what part in the Toxicology may be played by Nickel Carbonyl as such.*

In order to attack the problem on safe grounds, it was found necessary to ascertain how nickel carbonyl behaves when it comes into contact with the fluids and tissues of the body. This study involved the investigation of the following:

- (1) Under what conditions nickel carbonyl is dissociated.
- (2) What the solubility of nickel carbonyl in water at various temperatures and pressures is.
- (3) What the solubility in blood and in serum is.
- (4) Whether any compound of nickel carbonyl or nickel is formed in the blood, and
- (5) What becomes of the nickel in the body after any dissociation which may take place, has occurred.

*Vapour Tension of Nickel Carbonyl.*

The vapour tension of nickel carbonyl at temperatures between  $-9^{\circ}$  and  $+39^{\circ}$  C. was determined by Mond and Nasini (1891), Dewar and Jones (1903) and Mittasch (1902). The curves of these observers showed moderate agreement. It was, however, deemed advisable to redetermine the vapour pressure of the particular samples used in this investigation. The following method was employed. A tube was cleaned and closed at one end and then carefully dried. It was then filled with dry mercury and boiled out. The tube inverted over a bath of mercury was jacketed with water and a thermometer was also introduced into the jacket. A small tube provided with two stopcocks and with the lower end curved upwards for a few millimetres was then completely filled with nickel carbonyl, care being taken to exclude air in the lower portion. The lower curved end was then inserted under

the open end of the barometer tube and the lower tap opened. The nickel carbonyl was driven out of the small apparatus by warming the tube with the hand. The temperature was rapidly lowered to about  $3^{\circ}\text{C}$ . and then slowly taken up to  $35^{\circ}\text{C}$ . and again depressed to about  $5^{\circ}\text{C}$ . The vapour pressure at different temperatures was as follows:

Up values		Down values	
$2.85^{\circ}\text{C}$ .	167.61 mm.	$34.25^{\circ}\text{C}$ .	547.8 mm.
7.1	194.61	26.3	410.97
11.8	230.56	22.45	349.97
18.9	300.5	15.35	273.51
26.4	403.47	9.9	218.66
34.85	564.3	6.4	190.66

Fig. 1 is the curve interpolated from these values. The points determined by Dewar and Jones are given for comparison. The values determined at the lower and higher temperatures practically coincide, while the values in the middle of the curve (between  $19^{\circ}$  and  $24^{\circ}\text{C}$ .) are slightly lower than those determined by Dewar and Jones. The curve interpolated from these observers' determinations would therefore lie slightly higher and would be flatter.

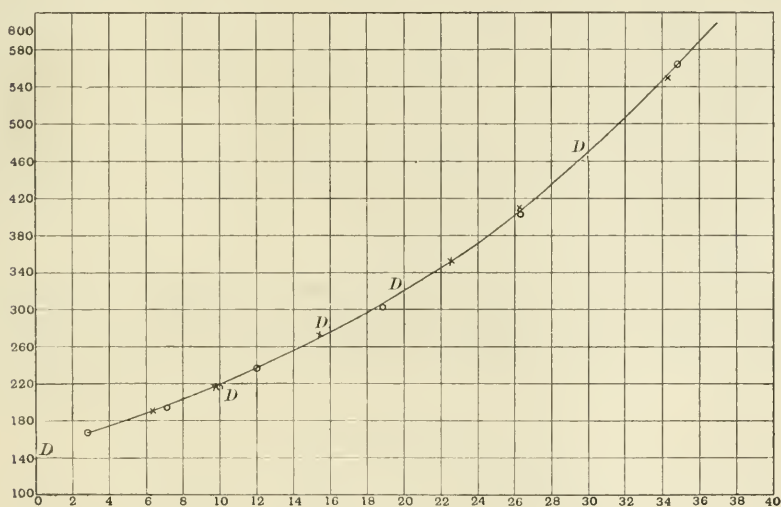


Fig. 1.

O = up values.

X = down values.

D = Dewar and Jones's points.

*Dissociation.*

When nickel carbonyl is kept in the presence of air and moisture, a deposit forms. This is due to the dissociation of the compound and the formation of a hydrate or carbonate of nickel.

In the burette containing nickel carbonyl, a deposit of hydrate or carbonate separates out after a few hours if any air is present. It is extremely difficult to keep liquid nickel carbonyl clear on account of this spontaneous dissociation, and the only safe way to avoid any deposit of hydrate is to fill the tube or burette with carbon monoxide after careful cleaning and drying.

Dewar (1903) noticed that when dissociation takes place out of contact with air and in the presence of an excess of carbon monoxide, recombination of part of the nickel with carbon monoxide occurs on cooling. In the vapour tension experiments, visible dissociation (*i.e.* black deposit on the tube) was seen at and above 22° C. Reversible action was noticed when a tube containing nickel carbonyl vapour and sealed at both ends, was heated at one spot. Under these circumstances a mirror of nickel is deposited on the glass, and at the periphery of the mirror a slight blackening is seen. This is most intense close to the edge of the mirror and diminishes imperceptibly further away from it. On cooling, some or all of this black deposit or even some of the mirror disappears again.

Some tubes of nickel carbonyl vapour with and without hydrogen were prepared and the dissociation watched. Evidence of reversible action was seen both in the tubes of pure nickel carbonyl and in those containing nickel carbonyl and hydrogen.

Slight dissociation was seen when tubes of nickel carbonyl vapour were kept in the water bath at 18° C. for several days. Dissociation takes place though slowly at lower temperatures. At room temperature (about 16° C.) a slight amount of black deposit was seen after 20 days in a flask containing water saturated with nickel carbonyl free from oxygen. At 35° C. nickel carbonyl dissociated fairly rapidly.

In an experiment dealing with the solubility of nickel carbonyl in water (to be described later) about 1.4 c.cm. of liquid nickel carbonyl was allowed to volatilise completely in an exhausted litre flask containing water at 35° C. Within 10 minutes a heavy black deposit of metallic nickel had separated out, and this increased steadily during the following two hours. The vapour pressure increased by 3 mm. in 10 minutes, and at the end of 20½ hours it had increased by 31 mm. from the carbon

monoxide set free. In a similar experiment, carried out at 23.4° C., enough nickel carbonyl was introduced into the flask to give a vapour pressure of 250 mm. After 20 minutes, the total pressure had increased by 11 mm.

A flask containing water, some mercury and an excess of nickel carbonyl, prepared anaerobically, was kept in the water bath at 29.7° C. After 14 days a black deposit had formed, in the fluid a thick layer was seen on the surface of the mercury and some black precipitate was adherent to the glass flask and capillary tubes.

When nickel carbonyl is evaporated into a large space containing air, a smokiness is seen, which may be due to floating particles of nickel hydrate or carbonate in extremely fine division.

#### *Solubility of Nickel Carbonyl in Water.*

The small solubility of nickel carbonyl and the fact that it dissociates so readily and also is apt to form an emulsion render it extremely difficult to determine the actual amount of nickel carbonyl taken up by water and aqueous fluids. The chief difficulties are due, firstly, to the fact that when nickel carbonyl in liquid condition is in excess, an emulsion is formed and the liquid does not settle readily, so that samples of the solution drawn off for examination frequently contain small amounts of liquid nickel carbonyl in suspension, and, secondly, that in dealing with partial pressures, dissociation takes place sufficiently extensively at temperatures above 10° C. to alter the pressure before equilibrium between the gases and the liquid has been obtained.

#### *Solubility at Constant Temperatures and Varying Pressures.*

The experiments were conducted at 9.8° C. The observations were made upon solutions which had been shaken with excess of liquid nickel carbonyl or had been saturated with gas at different partial pressures.

#### METHODS.

##### 1. *Experiments with Liquid Nickel Carbonyl in excess.*

A small flask similar to those employed for washing gases, but with tubes of capillary bore, and provided with stopcocks, was filled with boiled water, after having been washed out with hydrogen. The water was boiled in a large flask and carried over into the smaller flask by its own steam pressure. During the cooling process the tap in connection

with the longer tube was left open and connected with a capillary tube filled with mercury and dipping into a mercury bath. After the temperature had fallen to room temperature, the mercury and some water were sucked out through the tap in the short tube into an exhausted flask. Nickel carbonyl was then placed into a burette with two stopcocks, between two seals of mercury, and this burette having been applied to the longer tube (all tubes being filled with mercury) both the tap of this tube and the lower one of the burette were opened, and nickel carbonyl allowed to flow into the flask together with the mercury. The tap was then closed, the flask well shaken and kept at a constant temperature in the water bath. The flask was kept in the water bath for about 24 hours during the earlier experiments, but when it was found that complete solution occurred within one hour, the sojourn was shortened to this period. The flask was shaken at frequent intervals while in the bath.

In order to determine the amount of nickel carbonyl dissolved, a sample of the water was sucked out into a second gas washing bottle, which had been well washed out with hydrogen and evacuated. Before taking this sample, the flask was inverted and allowed to stand so that any nickel carbonyl in suspension might settle. A few c.cm. of water were first drawn off through the longer tube, so that, should any globules of nickel carbonyl be sticking to the inside of the capillary tube, these would first be got rid of. The weight of the water drawn off for examination was determined by weighing the second gas washing bottle before and after taking the sample.

The bottle was placed in connection with a hydrogen apparatus and a combustion tube, having two asbestos plugs. The combustion tube rested on two Ramsay burners, being supported by copper troughs with lids. The tube was thoroughly heated, after which hydrogen was bubbled slowly through the fluid to drive off the dissolved nickel carbonyl. The hydrogen was burnt at the further end of the tube. The heat dissociated the nickel carbonyl and caused a mirror of nickel to form on the combustion tube close to the Ramsay burner, while some of the remaining nickel was deposited in the asbestos plugs.

The flame at the pointed end of the combustion tube indicated if the least trace of nickel carbonyl escaped dissociation. This never took place when the tube was properly heated, provided that the hydrogen was bubbled through the solution slowly at the beginning. Toward the end of this stage, the test employed was to allow the tube to cool, while the hydrogen was turned off, and then on lighting the



hydrogen again, one could immediately detect if any nickel carbonyl was still present. The flame test is so delicate (detecting 1 volume in 400,000) that no appreciable error occurred in carrying it out.

After the carbonyl had been completely driven off, the nickel mirror in the tube and the nickel in the asbestos plugs were dissolved in nitric acid. The solution was evaporated to dryness, and the residue treated with hydrochloric acid, again evaporated and dissolved in water. The fluid was made up to a given bulk and the nickel determined by the colorimetric method with dimethylglyoxime, described by A. Harden and myself (1906).

## *2. Experiments in which Water was brought into equilibrium with the Gas at different Partial Pressures.*

A litre bolt-head flask with a special short neck was fitted with a rubber cork having five perforations. The perforations transmitted (1) a capillary tube dipping down to below the top level of the water, (2) a capillary tube dipping down to nearly the bottom of the flask, (3) a short tube to connect to the water pump, (4) a short tube to connect to the mercury gauge, and (5) a special burette for nickel carbonyl, with a three-way tap, through the one limb of which hydrogen could be let in.

The flask having been exhausted, the water was boiled (the mercury gauge being clamped off during this period to prevent distillation), any air given off was washed out by repeated displacement with hydrogen, and finally the flask was again exhausted. A measured quantity of nickel carbonyl was run into the flask from the burette and the flask was well shaken. A long tube containing mercury, which had been freed from water and air, was then attached to the capillary tube dipping to below the top level of the water. The sample was taken from the tube dipping nearly to the bottom of the flask and as it was being removed, mercury was let in, so that the upper level of the water was kept constant. The sample was dealt with as before.

One or two points in connection with the methods require especial attention. The hydrogen must be free from arsenic. Before displacing the nickel carbonyl, a blank test should be made each time to ensure that the rubber tubes connecting the parts of the apparatus (which should be as short as possible) have not retained any nickel carbonyl from a previous experiment dissolved in the rubber.

*Results.*

The following table gives the results obtained at 9.8° C. with varying pressures of nickel carbonyl:

V. P. in mm.	Mgrs. Ni per 100 grs. H <sub>2</sub> O		Calculated for 214 mm.*
214*	—	$\left\{ \begin{array}{l} 6.43 \\ 6.52 \\ 6.35 \end{array} \right\}$	6.43
186.5	5.53		6.34
138	3.92		6.09
183	5.64		6.59
109	3.32		6.32

\* Vapour tension of nickel carbonyl at 9.8° C.

The amount dissolved is directly proportional to the pressure, and these values plotted against the pressures as in Fig. 2 give a straight line.

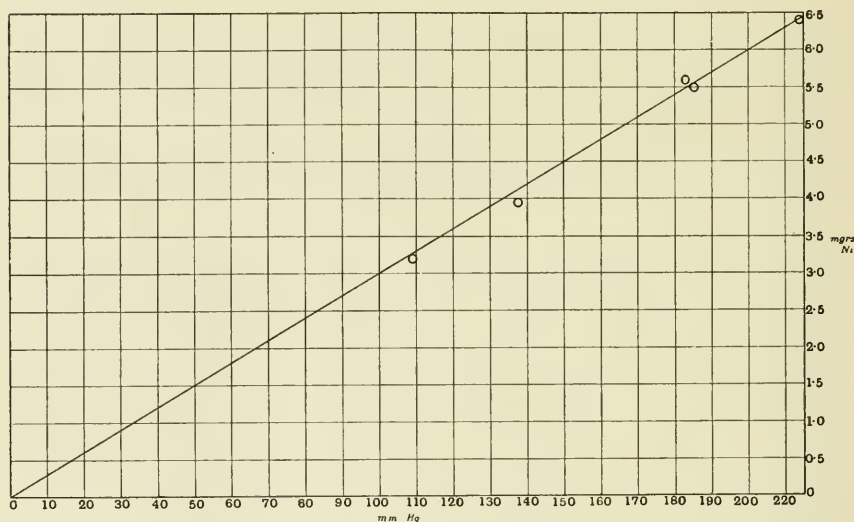


Fig. 2.

*Solubility of Nickel Carbonyl at Different Temperatures.*

For the determination of the solubility at varying temperatures, the method of saturating water with liquid nickel carbonyl and subsequently withdrawing a sample for analysis had to be employed for all temperatures above 10° C., owing to the quite appreciable dissociation of the gas which takes place leading to too high readings

of the partial pressure. The great disadvantage of employing excess of liquid carbonyl is the extreme precaution which has to be taken to avoid any minute particles of nickel carbonyl being carried over into the sampling flask.

The following results were obtained by the two methods employed :

1. Water shaken up with nickel carbonyl in excess.

Temperature	Mgrs. Ni per 100 grs.	Mgrs. Ni(CO) <sub>4</sub>	Ni(CO) <sub>4</sub> in c.cms.
9.8	$\left\{ \begin{array}{l} 6.42 \\ 6.52 \\ 6.35 \end{array} \right\}$	6.43	18.01
22.3	$\left\{ \begin{array}{l} 4.77 \\ 4.81 \\ 4.5 \end{array} \right\}$	4.69	13.63
29.1	$\left\{ \begin{array}{l} 4.41 \\ 3.61 \\ 3.45 \end{array} \right\}$	3.82	11.1
			1.45

2. Water brought into equilibrium with nickel carbonyl at various partial pressures.

Temp.	V. T. of Ni(CO) <sub>4</sub> at temp.	Part. P. of gas used	Mgrs. Ni per 100 grs. H <sub>2</sub> O	Calculated for V. T. at temp.
2.6	169	127	6.9	9.18
9.8	214	109	3.32	6.32
		138	3.92	6.09
		183	5.64	6.59
		186.5	5.53	6.34
23.4	363	250	2.13*	3.48
25	386	215.5	1.45*	2.6
35	564	132	0.38*	1.69
		199	0.48*	1.42
		235	0.7*	1.4

\* These results are certainly too low owing to dissociation.

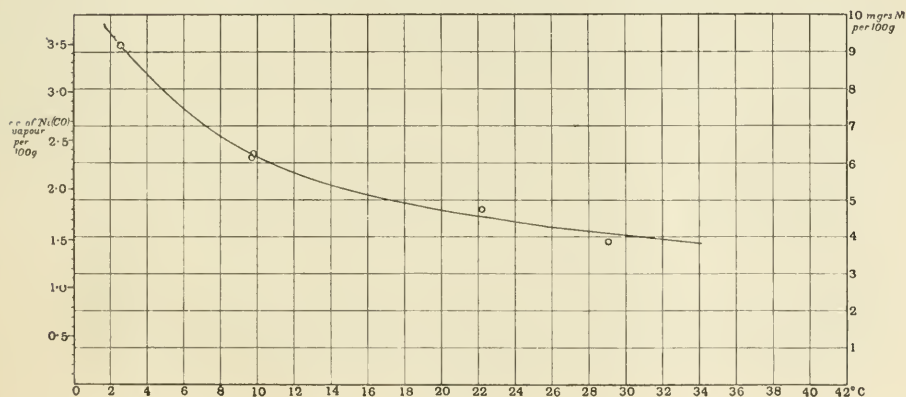


Fig. 3.

*Solubility of Nickel Carbonyl in Serum.*

The same methods as described above for water were attempted for serum, but it was found that in a viscid fluid the method with excess of liquid nickel carbonyl could not be relied on, since the inclusion of droplets of nickel carbonyl cannot be detected. The second method, therefore had to be resorted to. This, however, yields results which are too low at higher temperatures, on account of dissociation.

In displacing carbonyl from serum, a special gas washing bottle with the inside tube of capillary bore, only reaching to within a few millimetres above the level of the liquid, was used to prevent frothing. The displacement of the gas therefore took much longer than when hydrogen was bubbled through the liquid, and during this period small amounts of dissociation no doubt took place, as the temperature of the laboratory was between 15° and 20° C.

The results obtained are given in the following table and are set out graphically in Fig. 4.

*Solubility of Nickel Carbonyl at Different Temperatures.*

Temp.	V. T. of $\text{Ni(CO)}_4$ at temp.	Part. P. of gas used	Mgms. Ni per 100 grs. serum	Calculated for V. T. at temp.
1.1	157	112	16.1	22.57
10.0	219.5	139	10.07	15.71
23.5	364	186	4.66	9.0
35.0	568	209.5	2.71	6.81

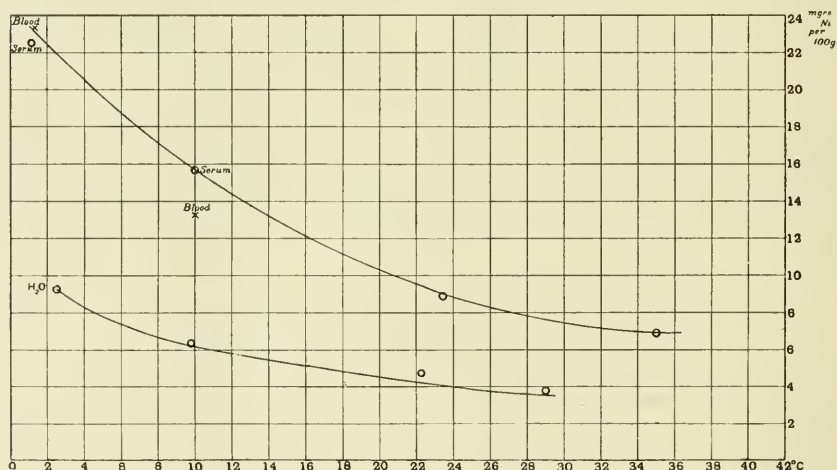


Fig. 4.

The solubility of nickel carbonyl in serum is higher than in water, and at body temperature the solubility in the former is about double that in the latter. In Fig. 4, the solubility curve in water is also plotted upon the same scale for comparison.

*Solubility of Nickel Carbonyl in Blood.*

At first it seemed not improbable that nickel carbonyl combined with the haemoglobin of the blood in virtue of its carbon monoxide groups, in which case the amount taken up by the blood would be greatly in excess of that dissolved in serum.

Langlois (1891) found that when blood was shaken up with nickel carbonyl the oxygen attached to the haemoglobin diminished by from 50% to 90%. He also injected rabbits with from 0.25 to 0.3 gr. of nickel carbonyl subcutaneously. The animals died within from 30 to 35 minutes. No oxygen was found attached to the haemoglobin after death. Lastly, he injected 0.3 gr. of nickel carbonyl into dogs, and obtained a great reduction of the oxyhaemoglobin. He therefore came to the conclusion that the oxygen was replaced by nickel carbonyl.

In an unpublished experiment, Shields was able to recover nickel carbonyl from blood by means of the gas pump. He concluded from this experiment that nickel carbonyl formed a chemical combination with a constituent of the blood.

In examining the behaviour of nickel carbonyl in blood it was thought advisable to proceed by carrying out parallel experiments with serum and blood. For this purpose, a litre bolt-head flask was fitted with a rubber cork, having four holes, to connect (1) with the filter pump, (2) with the mercury gauge, (3) with the nickel carbonyl burette and (4) with a T-shaped tube, leading to two equal 300 c.cm. flasks, one of which contained 100 c.cm. of blood and the other 100 c.cm. of serum. Each of the latter was provided with a capillary glass tube for removing samples of fluid. The flasks were exhausted, the serum and blood boiled vigorously *in vacuo* and the whole apparatus washed out four times with hydrogen, and again exhausted. Nickel carbonyl was run into the bolt-head flask until the mercury registered a pressure nearly equal to the vapour tension of nickel carbonyl at the temperature employed.

After a sufficient time had elapsed to ensure complete solution, samples of each were withdrawn into exhausted bottles and dealt with as in the previous experiments. While one sample was being analysed,



the other was kept at 3° C. in the cold room. As the displacing stage occupied some 2 to 2½ days, some dissociation possibly took place, even at the low temperature at which the flasks were kept. After the fluids were freed from nickel carbonyl, they were evaporated to dryness, ashed and the nickel content determined.

The results of two experiments are as follows:

1. Conducted at a temperature of 1.1° C.

Pressure of hydrogen before admitting Ni(CO)<sub>4</sub> ... 15.5 mm. Hg.  
Pressures when equilibrium was attained ... 127.5 mm.

Samples of the liquids were drawn off after 45 minutes. The nickel carbonyl in solution was determined as before.

The blood was dealt with first and yielded 17.35 mgrs. of nickel per 100 grs. which would correspond to 23.42 mgrs. of nickel per 100 grs. at the full vapour pressure of nickel carbonyl for the temperature employed.

The serum yielded 16.1 mgrs. nickel per 100 grs. or 22.57 mgrs. of nickel per 100 grs. calculated for the vapour pressure of nickel carbonyl at 1.1° C.

The blood further contained 2.64 mgrs. nickel in some form not displaceable with hydrogen and the serum contained 8.71 mgrs.

The following table expresses the result obtained, as mgrs. nickel per 100 grs.:

	Ni as Ni(CO) <sub>4</sub> mgrs.	Ni in solution mgrs.	Total mgrs. Ni
Serum	16.1	8.71	24.81
Blood	17.35	2.64	19.99

2. Conducted at a temperature of 10° C.

Pressure of hydrogen before admitting Ni(CO)<sub>4</sub> ... 25 mm. Hg.  
Pressure when equilibrium was attained ... 164 mm.

Samples were taken after 45 minutes. The serum was first dealt with, and the results were as follows:

	Ni as Ni(CO) <sub>4</sub> mgrs.	Ni in solution mgrs.	Total mgrs. Ni
Serum	10.07	8.27	18.24
Blood	8.53	11.32	19.85

Since the fluid dealt with first in each case gave higher results, it is probable that the amount of nickel carbonyl found in the second fluid was too low on account of dissociation having taken place. One is therefore justified in concluding that at 1.1° C. and 10° C., the amount

of nickel carbonyl taken up by blood is about the same as that by serum, and that the values, calculated for the full vapour pressure of nickel carbonyl at these temperatures, are about 23 and 15 mgrs. nickel per 100 grs. respectively. It is not obvious why the two results should be so nearly the same, for the physical and chemical properties of blood and serum differ very considerably.

It is therefore unnecessary to come to the conclusion, as Langlois and Shields have done, that a chemical combination between haemoglobin and nickel carbonyl is formed, as the total quantity of nickel carbonyl which blood is capable of taking up can exist in solution in serum. The oxygen of the haemoglobin is of course replaced by any carbon monoxide which may be liberated by dissociation of the nickel carbonyl. Langlois used quantities of nickel carbonyl which would yield sufficient carbon monoxide after dissociation to saturate the haemoglobin.

*On the Properties of the Substance formed by the Dissociation of Nickel Carbonyl in Water in the Presence of Air.*

When nickel carbonyl is dissolved in water and dissociation takes place, the condition of the nickel depends on the presence or absence of oxygen and carbon dioxide. In the entire absence of both of these, a precipitate of metallic nickel is formed, which can easily be removed by sedimentation or by filtration. When air is present, the nickel appears as a fine greenish white precipitate in a turbid fluid.

The precipitate is probably a hydrated sub-carbonate (Mond), but the exact composition has not been determined. The turbidity of solutions in which decomposition has occurred cannot be removed by passing the liquid through a paper filter, but when it is passed through a Berkefeld filter a clear filtrate is obtained, which, however, contains nickel in solution. The maximum quantity of nickel which was found in water after dissociation of nickel carbonyl had taken place and the carbonyl and undissolved salts had been got rid of by filtration through a Berkefeld filter was 3.47 mgrs. nickel per 100 c.cm. of water.

The properties of this nickel solution have been compared with those of nickel hydrate and carbonate. The differences are best understood by placing the behaviour of each side by side, and, for the sake of brevity, the product of dissociated nickel carbonyl will be referred to as *dissociation product*.

	Solubility	Filtration	Effect of heat	Effect of addition of Electrolytes (NaCl)
$\text{Ni}(\text{OH})_2$	Practically insoluble (less than $\frac{1}{1000}$ mgr. in 10 c.cm. of water).	When water is shaken up with $\text{Ni}(\text{OH})_2$ and passed through any filter paper, the filtrate is quite clear.	Unaltered as regards solubility after boiling.	No solution exists.
$\text{NiCO}_3$	Is soluble to the extent of 0.48 mgr. Nickel per 100 c.cm. of water at room temperature (18 to 23°C.).	When water is shaken up with Nickel Carbonate, the filtrate is only clear if a close filter is used.	Boiling deposits a trace of precipitate containing Nickel. The filtrate contains the greater bulk.	Electrolytes do not precipitate the salt from solution.
<i>Dissociation Product</i>	Is dissolved to the extent represented by 3.47 mgrs. Nickel per 100 c.cm. water at room temperature.	When water is shaken up with Nickel Carbonyl and the Carbonyl is removed, the liquid can only be cleared of its turbidity by passing through a Berkefeld filter.	From the solution of <i>Dissociation Product</i> , the Nickel is almost completely precipitated by boiling. The precipitate can be completely removed by filtering through paper, leaving the merest trace of Nickel in solution.	The addition of Electrolytes to solution of <i>Dissociation Product</i> precipitates some of the Nickel. When a saturated solution of NaCl is added, about 25% of the Ni is precipitated, while when $(\text{NH}_4)_2\text{SO}_4$ is added, rather less is thrown out of solution.

Nickel hydrate is slightly soluble in its mother liquor, *i.e.* sodium hydrate; nickel carbonate is more soluble in its mother liquor, *i.e.* sodium carbonate, and the fluid becomes slightly turbid, which turbidity can only be removed by filtration through a close filter paper.

When carbonic acid is passed through a solution of nickel carbonate, and the liquid filtered through a close filter paper, the solubility is found to be slightly diminished (0.41 mgr. against 0.48 mgr. nickel per 100 c.cm. of water).

After standing for a few days, the solution of *dissociation product* becomes opalescent, and after the 5th day or later a fine precipitate separates out. This precipitate contains nickel.

The solution of *dissociation product* was subjected to dialysis and it was found to pass through the membrane in 36 hours. 74 c.cm. of a solution containing 1.1 mgr. nickel per 100 c.cm. were placed inside the skin and 1500 c.cm. of water were placed outside. The dialysate contained 0.96 mgr. of nickel. A solution of *dissociation product* containing 0.15 mgr. in 50 c.cm. was dialysed for 12 hours with 800 c.cm. of water. At the same time, a solution of nickel carbonate of the same strength

was also subjected to the same procedure. The latter was found to have dialysed completely, while the dialysis in the former was not complete. The dialysate contained 0.03 mgr. of nickel, but the fluid inside the membrane only contained 0.01 mgr., so that the remainder was absorbed by the membrane. A solution of *dissociation product* containing 0.41 mgr. per 100 c.cm. was next dialysed for seven hours. It was found that roughly one-third of the same strength solution of nickel carbonate passed through the membrane in this time, the rest being found in the fluid inside the skin. In the case of the *dissociation product* although about 0.02 mgr. of nickel was still in the fluid in the skin, only a mere trace could be found in the dialysate. The greater part of the nickel had therefore been absorbed into the membrane.

*Solubility of Dissociation Product in Serum and its Relation to the Proteid Content.*

Serum was saturated at 35° C. with nickel carbonyl (*a*) in the absence of air and (*b*) in the presence of air, and was then subjected to various investigations. The serum in the former case was boiled *in vacuo*, but this is insufficient to remove all the carbon dioxide. The nickel carbonyl having been removed, the serum was filtered through a Berkefeld filter. The resulting filtrate was quite clear, but the colour varied in consequence of concentration behind the filter.

1. Experiments conducted with serum which had been boiled *in vacuo* and the oxygen removed.

Various samples were analysed for nickel and also for nitrogen (Kjeldahl).

The results were as follows:

	Mgrs. Ni per 100 grs. serum	N per 5 c.cm., expressed as $\frac{n}{10}$ NH <sub>3</sub>
1.	2.98	13.7
2.	3.1	18.7
3.	2.18	14.7
4.	1.3	4.7
5.	2.48 (?)	5.2

The figures show no proportion between nickel and nitrogen, so that a chemical compound is excluded.

2. Experiments conducted in the presence of air.

Serum, serum diluted with water, and water were saturated with nickel carbonyl, kept at 35° C. for one hour and then placed in the cold room for 20 hours. The nickel carbonyl was driven off by bubbling air

through the liquids, and the liquids were passed through a Berkefeld filter. Samples ashed gave the following values.

1. Pure Serum	contained	13.33 mgrs. Ni per 100 grs.		
2. Serum + water (1:1)	„	7.75	„	„
3. „ „ (1:2)	„	7.25	„	„
4. „ „ (1:4)	„	6.19	„	„
5. Pure water	„	0.72	„	„

Here again no ratio between nickel and nitrogen (dilution of serum) is to be found.

### *Dialysis.*

Serum treated as before in the absence of oxygen was subjected to dialysis for 24 hours.

- 15 grams of Serum inside the skin and 1400 c.cm. H<sub>2</sub>O outside.  
Dialysate contained 0.3 mgrs. Nickel.  
Fluid inside the skin „ 1.4 „
- 25 c.cm. of Serum and 1315 c.cm. Water.  
Dialysate contained 1.57 mgrs. Nickel.  
Fluid inside the skin „ 0.63 „
- 40 c.cm. of Serum and 1500 c.cm. of Water.  
Dialysate contained 2.65 mgrs. Nickel.  
Fluid inside the skin „ 1.22 „

The serum in 2 and 3 was not quite fresh and allowed some coagulable material to pass through the membrane, but the results show that the nickel is only partially dialysable.

### *Discussion of the Condition in which Nickel exists in Serum after Dissociation of Nickel Carbonyl has taken place.*

The nickel derived from the dissociation of nickel carbonyl in serum in the presence of oxygen and carbon dioxide exists partly in solution and partly as a finely divided precipitate. The latter can only be removed by filtering through a Berkefeld filter. The dissolved nickel does not stand in any proportion to the nitrogen of the serum. The maximum quantity found dissolved in serum expressed as nickel was 13.33 mgrs. per 100 grs. of serum. Solutions of this *dissociation product* are only partly dialysable.

The increased solubility in serum as against water must depend either on the presence of inorganic and organic salts, or on the presence of proteid and other colloidal matter (*e.g.* pigment) or both.

In order to decide to what conditions this increased solubility is



due, experiments with nickel hydrate, nickel carbonate and *dissociation product* in various solvents were carried out.

Serum was filtered through a Martin Gelatin filter, and a filtrate was obtained which practically represented the salts of serum dissolved in the water of serum. 100 c.cm. of this solution dissolved 5.04 mgrs. of nickel as *dissociation product*. The same quantity of the solution dissolved 4.15 mgrs. of nickel in the form of nickel carbonate.

The solutions in the salts of serum solution however became turbid after two days. The turbidity could not be removed by filtration through the closest filter paper.

A fresh solution of sodium phosphate ( $\text{Na}_2\text{HPO}_4 + 12\text{H}_2\text{O}$ ) containing 0.25 gr. to the litre was next used, as representing the minimum value of phosphates in serum (Hammersten gives this at 0.05 gram pentoxide of phosphorus ( $\text{P}_2\text{O}_5$ ) per litre). The solution was saturated with carbon dioxide to correspond more nearly to the condition of serum. It dissolved 2.45 mgrs. of nickel as *dissociation product* and 12.3 mgrs. of nickel as nickel hydrate per 100 c.cm.

Nickel carbonate was also shaken up in the phosphate solution and was found to be practically insoluble.

The solubility of *dissociation product* in solutions of sodium carbonate is approximately the same as that in water.

Since serum dissolves *dissociation product* to the extent of 13.3 mgrs. of nickel per 100 grs. of serum, and whereas water solutions of phosphate of sodium and solutions of the salts of serum dissolve considerably less, there is a balance of dissolved nickel yet to be accounted for.

Serum dissolves freshly precipitated nickel hydrate.

Nickel hydrate freshly precipitated was added to serum, well shaken up at room temperature and filtered through a close filter paper. The filtrate which was quite clear contained 22.5 mgrs. of nickel per 100 c.cm. The serum was not freed from oxygen or carbon dioxide. The unashed filtrate gave the dimethylglyoxime reaction. Since serum to which nickel sulphate and an excess of potassium hydrate are added (*i.e.* potassium nickel albuminate) does not give this reaction, one can deduce that the nickel therefore existed in the filtrate as free ions.

The solubilities of nickel hydrate, nickel carbonate and *dissociation product* are given in the following table for comparison. The solubility of *dissociation product* is inconstant, and depends upon the temperature at which the nickel carbonyl is dissociated. The lower the temperature

at which dissociation takes place, the greater is the amount of material which goes into solution in the water.

The figures given are therefore only to be regarded as maximum values obtained.

	H <sub>2</sub> O	Na <sub>2</sub> HPO <sub>4</sub>	Salts of serum sol.	Serum
Ni(OH) <sub>2</sub>	0	12·3	—	22·5
NiCO <sub>3</sub>	0·48	0	4·5	16·03
<i>Dissociation Product</i>	3·47	2·45	5·04	13·3

It was thought that if the proteid material combined with nickel, this compound might be thrown down by precipitating the proteids with ammonium sulphate.

25 c.cm. of serum in which *dissociation product* was dissolved was used. Ammonium sulphate was added to this to produce a 33% saturation. The precipitate was collected and ashed and the ashed material is called (a) below. Next, sufficient ammonium sulphate was added to the filtrate to produce a 50% saturation. The ashed precipitate is called (b). The filtrate was saturated with solid ammonium sulphate. The precipitate ashed is called (c). The filtrate of this was boiled and the precipitate and filtrate ashed are called (d) and (e) respectively.

(a)	Containing	0·12 mgrs. Ni or 0·49 mgrs. Ni per 100 grs.
(b)	„ about	0·01 „ or 0·04 „ „ „
(c)	„	0 or 0
(d)	„	0 or 0
(e)	„	1·27 „ or 5·1 „ „ „

This experiment was further compared with the following:

Serum was shaken up with nickel carbonate and filtered through a close filter paper. The filtrate was quite clear. Ammonium sulphate was then added as before.

The results were as follows:

(a)	Contained	6·7 mgrs. Nickel per 100 grams Serum.
(b)	„	4·7 „ „ „ „
(c)	„	0·9 „ „ „ „
(d)	„	0 „ „ „ „
(e)	„	4·9 „ „ „ „

In the case of *dissociation product* the small fraction thrown down may have been due to the action of electrolytes, but the reason why nine-tenths should remain in solution is not clear. In the second case, while the action of electrolytes cannot be excluded, the results seem rather to suggest that a part of the nickel had entered in combination with the proteid.

The facts that when in aqueous solution, nickel is precipitated by boiling, and by adding electrolytes, and that at a certain stage of dialysis, adsorption into the membrane takes place, and also that the difficulty of obtaining a constant strength of the aqueous solutions may be due to the substance not being in ordinary solution, are strongly in favour of the view that *dissociation product* passes into colloidal solution.

The increased solubility of *dissociation product* in serum can also be explained on this view. The influence of serum in keeping metallic precipitates in colloidal solution is well known. When copper sulphide, for example, is formed in serum, it does not precipitate in the usual way but forms a colloidal solution.

The increased solubility of *dissociation product* in serum can be explained in this way. At present, however, it is impossible to exclude the possibility of a proteid combination, but this would at most only affect a small proportion of the dissolved nickel. That the major part of the nickel is not combined with the proteid has already been shown.

To sum up, nickel carbonyl dissociates in the presence of oxygen and carbon dioxide into carbon monoxide and a nickel compound, which is probably a hydrated basic carbonate. This substance is slightly soluble in water (0.0035 %) and considerably more soluble in serum (0.0133 %). When this substance is formed in water or in serum it exists partly in a state of extremely fine division, and this causes a turbidity of the liquid to appear. The particles are so small that filtration through a Berkefeld filter is necessary to clear the liquid.

A comparison with nickel hydrate and nickel carbonate shows that the substance obtained from the dissociation of nickel carbonyl is not identical with either of these, as they exist when precipitated in the ordinary way. The solutions in water and in serum appear to be colloidal solutions. From the aqueous solutions the substance can be precipitated by heat, and to a certain extent by the action of electrolytes, and a precipitate forms spontaneously after standing for some days.

The solubility of this substance in solutions of the salts of serum and in solutions of sodium phosphate does not account for its relatively high solubility in serum. There is no evidence of the existence of a proteid combination.

## SUMMARY AND CONCLUSIONS.

1. Nickel carbonyl is a highly toxic compound.
2. Dissociation of nickel carbonyl takes place rapidly at the temperature of the body in the presence of air, moisture and carbon dioxide.

Nickel carbonyl also dissociates at the temperature of the body in the absence of air, moisture and carbon dioxide, but not to the same extent as in the presence of these owing to the fact that a reversible action occurs between the nickel and the dissociated carbon monoxide.

3. Nickel carbonyl is soluble in water to the amount of 2.36 c.cm. of vapour (*i.e.* 6.43 mgrs. of nickel) per 100 grs. at 9.8° C.

The amount dissolved in water is directly proportional to the pressure, when the temperature is constant.

The solubility in water diminishes as the temperature is raised.

4. The solubility of nickel carbonyl in serum and in blood is about 2½ times greater than that in water. At 10° C. 100 grs. of serum dissolves 5.98 c.cm. of nickel carbonyl vapour (*i.e.* 15.71 mgrs. of nickel).

5. When nickel carbonyl is brought into contact with water, serum, blood and other liquids, if oxygen and carbon dioxide are present, dissociation occurs and a substance is formed, probably a hydrated basic carbonate of nickel, which is slightly soluble, but also forms a very fine precipitate, which renders the liquid turbid. The liquid can only be cleared of the turbidity by passing it through a Berkefeld filter.

6. Water dissolves about 0.0035 % of the product of dissociated nickel carbonyl (reckoned as nickel) at 18° C. About the same amount is dissolved in 10 % solutions of sodium carbonate.

The solubility of this product of dissociated nickel carbonyl is greater in serum than in water. Serum dissolves about 0.0183 % (reckoned as nickel). A solution of the salts of serum, under the same conditions, dissolves 0.005 % (reckoned as nickel). Solutions of sodium phosphate of approximately the same strength as the phosphates in serum, dissolve 0.0025 % (reckoned as nickel).

7. Nickel hydrate is insoluble in water but is soluble in serum to the extent of 0.0225 gr. nickel per 100 grs. serum, and in sodium phosphate solutions to the extent of 0.012 % (reckoned as nickel).

8. Nickel carbonate is soluble in water to the extent of 0.0005 % (reckoned as nickel), and in serum to an amount, corresponding to 0.016 gr. % of nickel. Salts of serum dissolve 0.0045 gr. % of

nickel carbonate reckoned as nickel. Nickel carbonate is insoluble in sodium phosphate solutions.

9. *Dissociation product* in aqueous solution is precipitated by boiling, and to a certain extent by the addition of electrolytes. Some of it is thrown out of solution spontaneously on standing for some days, whereas solutions of nickel carbonate are not precipitated by either of the above means.

10. Solutions of *dissociation product* dialyse more slowly than solutions of similar strength of nickel carbonate, and at a certain stage in the dialysis the greater part of the nickel is adsorbed into the membrane.

11. No constant ratio exists between the nickel and the nitrogen contents of solutions of *dissociation product* in serum of different strengths.

12. When the proteids of the serum, containing the product of dissociated nickel carbonyl in solution, are precipitated by ammonium sulphate, nine-tenths of the nickel remains in solution. One-tenth is precipitated by adding sufficient ammonium sulphate to produce one-third of saturation, and about an additional one-hundredth by producing one-half of saturation.

13. The product of dissociated nickel carbonyl when dissolved in serum is only incompletely removable from solution by dialysis.

The product of dissociated nickel carbonyl is therefore not identical with nickel carbonate or nickel hydrate, and appears to exist in a condition of colloidal solution.

14. The poisonous properties of nickel carbonyl do not depend, as was at first supposed, on the carbon monoxide of the compound.

15. Nickel carbonyl when mixed with air and inhaled by an animal, cannot be absorbed as such, as it becomes split up into the nickel containing substance (?hydrated basic carbonate of nickel) and carbon monoxide, before or soon after reaching the alveoli of the lungs.

16. The poisonous effects of nickel carbonyl are entirely due to the nickel of the compound. The peculiar toxicity of the compound is due to the fact that being introduced in a gaseous form and that the nickel is deposited as a slightly soluble compound in a very fine state of subdivision over the immense area of the respiratory surface.

In Part II. the passage of the nickel through the body from its deposition on the respiratory surface to its ultimate excretion will be followed out.



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A CYSTIC DISEASE OF THE HEART, GIZZARD AND  
MUSCLES OF YOUNG GRASS PARAKEETS (*PSITTACUS*  
*UNDULATUS*) DUE TO A PROTOZOON PARASITE.

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(Plates XIII and XIV.)

VARIOUS species of the Order Sarcosporidia, some of which have been studied with great care, infest the muscles of different kinds of birds and mammals. Muscle parasites resembling the one described below do not, however, appear to have been described.

Owing to the failure of attempts to transmit the disease and the limited amount of material these observations are very incomplete, but as the disease is peculiar in the appearance of the cysts and in their distribution, and as no indications have been obtained as to the mode of infection or fate of the parasites, it was thought that a brief description might call the attention of those, who have the opportunity of obtaining material, to its existence.

Two young parakeets which were bred in captivity from apparently healthy parents were sent to me for examination. The birds had never left their nests, and it was thought that their death might be due to neglect by the parents.

On examination parakeet No. 1 showed on the heart wall a large collection of small, closely packed cysts (Pl. XIII, Fig. 5) as well as several smaller groups and scattered single cysts. On teasing portions of the heart muscle numerous cysts were found within the muscle substance. Numerous cysts were also found on the surface and within the muscle of the gizzard, and a few were discerned in the pectoral muscles. No lesions either macroscopic or microscopic were found in the other organs, cavities or blood. In the second specimen (No. 2) large numbers of cysts

were found in the gizzard, but none in the heart or muscles. No other lesions were observed.

Some time later another specimen (No. 3) was received. This bird was very young and the body was somewhat decomposed. In the gizzard very large numbers of cysts, occurring in masses which could be recognised by their yellow colour were found. None were found microscopically in the heart or muscles.

Another specimen showed no cysts.

#### *Macroscopical appearances.*

On the surface of the heart, just beneath the visceral pericardium, (No. 1) and on the surface of the gizzard (Nos. 1, 2 and 3) the cysts were found in large and small groups, as raised yellowish areas. Single cysts were also common. Within the muscular substance they were also found in groups, but in this situation there was a tendency for the cysts to be arranged in rows of three or more individuals. With the aid of a needle the cysts could usually be easily separated from the muscle substance, and isolated as small, round, smooth masses, varying in diameter from .5 mm. to scarcely visible particles.

#### *Microscopical examination of sections.*

Sections through the affected areas showed numerous cysts, but except in one instance (Pl. XIV, Fig. 6) no acute inflammatory reaction was ever observed round them. All the cysts were of comparatively large size, and since no young forms were noticed, it is difficult to ascertain whether they were originally formed within the muscle fibres or not. Usually single cysts and groups are surrounded by a delicate zone of fibrous tissue, but occasionally a more extensive fibrous tissue capsule can be seen. Delicate strands of fibrous tissue sometimes break up the muscle fibres immediately surrounding the cysts.

In the larger groups many of the cysts are in contact with one another (Pl. XIII, Fig. 1), and the same may be the case when three or four cysts occur in a row (Pl. XIII, Fig. 4). At other times the members of a group are separated by small quantities of muscle or fibrous tissue (Pl. XIII, Fig. 2).

The conformation of the walls and the contents of the cysts show great variations. In every case the wall of the cyst consists of a thick, smooth membrane in which no radial striation or other structure has been discovered. This membrane, judging by its appearance in collapsed cysts, is very elastic (Pl. XIII, Fig. 4, Pl. XIV, Figs. 8, 9, 10).



I. In some cysts this membrane is complete and closely adherent to the delicate fibrous layer surrounding it. Within the membrane there is often a thin layer of finely granular material. The rest of the cyst is filled with a mass of partially or completely separated spores, apparently imbedded in a small quantity of supporting material.

II. Cysts of the type just described frequently rupture and discharge their contents. Pl. XIII, Fig. 3, (A) shows a cyst in the act of rupture with a large mass of spores passing out. The same figure (B) also shows another cyst which has ruptured. The membrane has become detached from the surrounding muscle tissue and has collapsed. In this case the spores are lying round the collapsed membrane.

Pl. XIV, Fig. 10 shows another cyst in a similar condition. The membrane has collapsed and the rupture is distinctly seen (A). In this case some of the spores are still lying within the membrane, and others round it, but large numbers can also be seen between the muscle fibres (B) at a considerable distance from the remains of the cyst. In some cases the spores have been traced in the connective tissue for long distances and more rarely masses of spores may be seen in the vessels (Pl. XIV, Fig. 7 (A)). In a few cases small extravasations of blood have been seen in which spores are mixed with the blood corpuscles.

III. In other cases the cysts partially or wholly discharge their contents without collapse of the membrane. Such empty cysts are generally filled with blood corpuscles, amongst which a few spores can often be found (Pl. XIII, Fig. 2 (A, B)). Occasionally a row of cysts filled with blood communicate by distinct openings with one another. Round these cysts, especially opposite small openings in the membrane, collections of spores can often be found.

IV. Cysts which do not rupture may undergo other changes, which are difficult to interpret, but apparently end in the contraction of the cysts into small hard irregular masses. From a study of the sections it appears probable that the changes take place in the following manner.

(a) The material in which the spores are embedded seems to contract, resulting in the production of irregular slit-like spaces between which the closely packed spores lie (Pl. XIII, Fig. 4 (A), Pl. XIV, Fig. 6) surrounded by a matrix arranged in irregular columns. (b) Further contraction leads to the widening of these spaces and partial detachment of the membrane from the surrounding muscle (Pl. XIII, Fig. 2 (C)). A striking example is shown in Pl. XIV, Fig. 9. At this time the spores are very closely packed together and can only be distinguished with great difficulty as darkly staining granules in a



moderately darkly staining matrix, arranged in very irregular columns. (c) Later further contraction takes place and the membrane is detached from the muscle over large areas and becomes greatly wrinkled (Pl. XIII, Fig. 4 (D)). As no support is now afforded by the lax membrane the slits collapse and the mass forming the interior of the cyst presents an almost solid appearance, and no spores can be distinguished. (d) Still later owing to further contraction the mass becomes almost separated from the membrane being only attached to it by a number of thin trabeculae (Pl. XIV, Fig. 8).

At this stage the spaces seem to be filled by serious exudation.

(e) Finally further contraction leads to the formation of irregular, darkly staining bodies surrounded by the distorted remains of the membrane (Pl. XIV, Fig. 11), some of which are reduced to very small dimensions.

#### *Spores.*

A large number of smears made from the contents of cysts at various stages were examined after staining by Giemsa's and Leishman's methods.

In some cases masses of minute, irregular, red stained bodies were found imbedded in faintly staining blue material. This condition apparently represents very young spores incompletely separated from one another (Pl. XIV, Fig. 12 (A)). In other cases small, discrete bodies were found each consisting of a small mass of chromatin staining material surrounded by a thin zone of blue staining protoplasm (Pl. XIV, Fig. 12). The chromatin was sometimes dense and rounded in outline, and sometimes irregularly shaped and contained darker staining points. This condition seems to represent an early stage in the separation of the spores. In thick smears small masses of spores sometimes seem to be arranged in groups, but no spore morulae with a definite cell wall as in the pansporoblasts of *Rhinosporidium kinealyi* are present<sup>1</sup>. In other cases the spores consist of rounded or oval masses which stain a deep red colour with Giemsa's stain. Sometimes the staining is uniform, but at other times one or more very minute more darkly staining areas may be present. Some of these bodies are surrounded by a very thin zone of blue, but others show no trace of it (Pl. XIV, Fig. 13). Traces of blue staining material are to be found between these bodies. This appears to be the most advanced stage of spore

<sup>1</sup> J. M. Beattie (1906). *Rhinosporidium kinealyi*; a sporozoon of the nasal mucous membrane. *Journ. of Pathol. and Bacteriol.* Vol. XI. p. 270.

formation met with in these cysts. Although in the preparation of the smears some of the spores became distorted and elongated, in no case were large numbers of elongated or falciform bodies seen.

Fresh preparations showed only minute, round, motionless bodies.

#### *Staining reactions.*

The cyst membrane takes on a well marked yellow colour when stained by Van Gieson's method, and a reddish colour by Leishman's stain. It stains very poorly by haematoxylin, carmine or thionin. In sections the spores are stained red by Leishman's stain and can be readily detected in the cysts when stained by haematoxylin or methylene blue. The contents of the contracting cysts take up the common dyes very readily.

#### *Infection experiments.*

The contents of about 100 crushed cysts were injected into the abdominal cavity of an adult parakeet, and another bird ate a large number of cysts. Although carefully watched for eight months these birds never showed any symptoms. Finally they were killed and careful autopsies made. No lesions were found.

So far as I am aware no disease resembling this one has been described. The cysts appear to be produced by a protozoan parasite during a stage in its life history. The constant presence of the cysts in the gizzard suggests that the birds are infected by feeding, and that the other muscles are infected secondarily. On the other hand, since the spores sometimes get into the blood, it is possible that the disease may be conveyed by external blood-sucking parasites. No indication has been obtained as to how the parasites get into the food, if infection occurs through feeding, or of the ultimate fate of the spores which escape from the cysts. It is interesting to note that though only young nestlings died from the disease no young cysts were encountered, and many were found which had become completely contracted.

Though differing in many respects this parasite more closely resembles *Rhinosporidium linealyi* than any other cyst producing protozoon.

### DESCRIPTION OF PLATES.

#### PLATE XIII.

Fig. 1. A section through the large mass of cysts on the heart of parakeet No. 1 (Fig. 5). Large numbers of cysts can be seen with very little tissue separating them. Some are full of spores, others contain blood and others have degenerated.

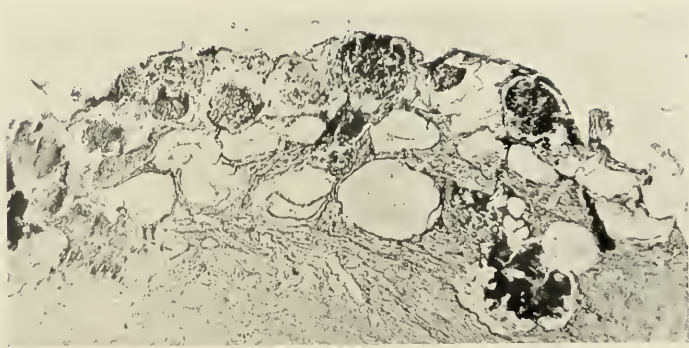


Fig. 1.

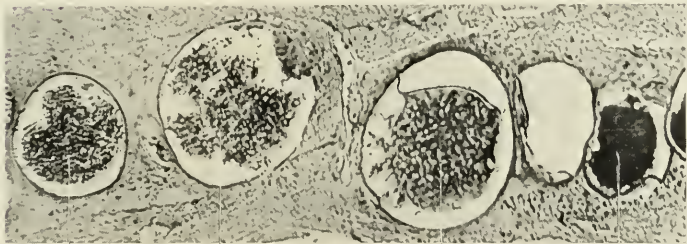


Fig. 2.

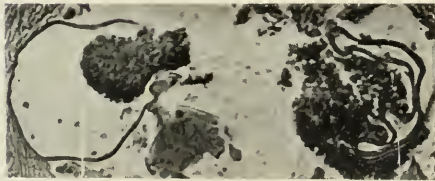


Fig. 3.

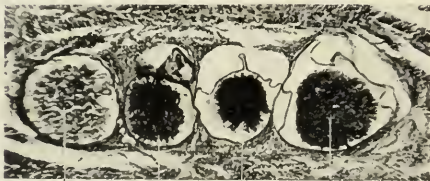


Fig. 4.



Fig. 5.





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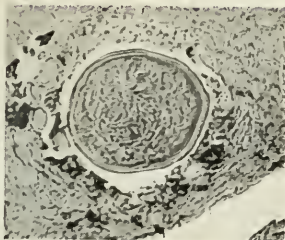
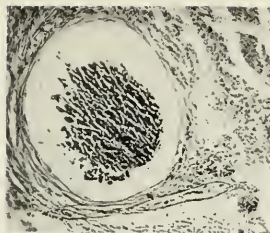


Fig. 6.



A B

Fig. 7.

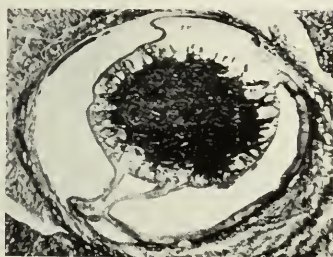
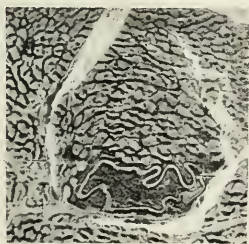


Fig. 8.



Fig. 9.

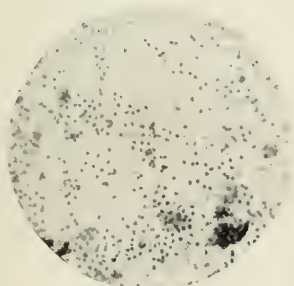


B A

Fig. 10.



Fig. 11.



A A

Fig. 12.



Fig. 13.





- Fig. 2. A row of cysts from the gizzard (Bird No. 1). Two (A and B) contain blood corpuscles and a few spores; another (C) is in an early stage of degeneration and shows slit-like spaces in its contents. Over a quarter of its circumference the membrane has become detached from the muscle. Cyst D is in an advanced stage of degeneration and contains a solid black mass.  $\times 150$ .
- Fig. 3. Cysts from the gizzard (Bird No. 2) during and after rupture of the membrane. Cyst A is in the act of discharging a mass of spores. Cyst B has ruptured and the membrane is distorted. The spores are lying round the distorted membrane.  $\times 150$ .
- Fig. 4. A row of cysts from the gizzard (Bird No. 1). Cyst A is in an early stage of contraction. It shows slit-like spaces, but the membrane is only wrinkled and is not detached from the muscle. Cyst D is in a slightly more advanced condition of contraction. The slits are much smaller, and the membrane has become detached over large areas. Cyst B shows an intermediate condition between A and D. Cyst C is still more contracted.  $\times 90$ .
- Fig. 5. Photograph of a water colour drawing of the heart of bird No. 1. It shows one very large collection (A), two medium sized collections (B) and several small groups (C) of cysts.

## PLATE XIV.

- Fig. 6. A cyst in an early stage of contraction in the pectoral muscle (Bird No. 1). The irregular slit-like clear spaces are well seen. Some inflammatory cells can be seen round the cyst.  $\times 150$ .
- Fig. 7. A mature cyst containing a mass of spores (heart, Bird No. 1). In the small vessel (A) an elongated mass of spores can be seen. A large collection of spores (B) is also to be seen between the muscle fibres.  $\times 100$ .
- Fig. 8. A contracting cyst from the gizzard (Bird No. 2). The contents show narrow irregular slits in the centre and the main mass is connected to the membrane by numerous fine trabeculae. The membrane has become detached from the muscle over large areas.  $\times 135$ .
- Fig. 9. A contracting cyst from the gizzard (Bird No. 1) showing a peculiar condition. The central mass has become separated from the membrane over half the circumference of the cyst. The contents have undergone contraction resulting in the production of numerous large irregular spaces. Spores could still be distinguished with difficulty.  $\times 150$ .
- Fig. 10. Remains of a ruptured cyst in the pectoral muscle (Bird No. 1). The membrane has collapsed and shows a rupture (A). Large numbers of spores are seen within and immediately round the membrane, but small masses (B) have been forced some distance from the cyst.  $\times 100$ .
- Fig. 11. A cyst in an advanced condition of contraction from the heart (Bird No. 1). The distorted remains of the membrane can be seen round the dark contents.  $\times 200$ .
- Fig. 12. Spores in a smear preparation of the contents of a cyst. The majority of the spores are separate, each showing a round or slightly irregular mass of chromatin surrounded by pale protoplasm. Several masses (A) of partially separated spores can be seen. Stained by Giemsa's method.  $\times 1000$ .
- Fig. 13. Spores in a smear preparation of the contents of a cyst. In this case the spores are completely separated and appear as oval or round almost uniformly staining chromatin masses. Some remains of the supporting material can be seen. Stained by Giemsa's method.  $\times 1000$ .

## EXPERIMENTAL INVESTIGATIONS ON FRAMBOESIA TROPICA (YAWS).

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(Plates XV and XVI and One Figure.)

IN this paper I propose to record briefly the results of my investigations on the inoculation of framboesia in the lower animals and other experimental researches on the disease.

### *Inoculation of Yaws in Monkeys.*

My first experiments, made at the beginning of 1905, on a "purple-faced monkey" (*Semnopithecus cephalopterus*) were negative. In February and March of 1906 I inoculated three monkeys of the genus *Macacus*, with positive results in one case. The monkey which was successfully inoculated with yaws was later inoculated with syphilis with positive results. In the meantime Neisser, Baerman and Halberstädter (1906) published a report on their results in the inoculation of yaws in monkeys, coming to the conclusion that monkeys of a high as well as those of a low type are susceptible to infection with yaws; and that monkeys immunized against syphilis do not become immune against yaws. I have continued the investigation on numerous monkeys of the genus *Macacus* and *Semnopithecus*. In both genera the positive results are fairly numerous, provided the scarifications on which the yaws material is inoculated are made as deep as possible. I quote two of the experiments which gave positive results.

*Monkey No. 4 (Macacus pileatus).* 10. XI. 06. The scrapings taken from a non-ulcerated yaws papule were thoroughly rubbed into the scarified spots over the left eyebrow. The slight local inflammatory reaction caused by the scarification subsided in three days. Nineteen days after the inoculation a very small flattened



Fig. 1.

Figs. 1 & 2. Experimental Yaws.



Fig. 2.

Framboetic lesion at the place of inoculation (eyebrows) in monkeys.





papule, surrounded by an infiltrated zone, appeared at the seat of inoculation. The lesion soon became enlarged and moist, the secretion drying into a thick crust. On removing this crust a granulating raw surface was seen. Two months later, the first lesion being still present, four more papules appeared, two on the lower part of the forehead, close to the primary lesion, and two just over the upper lip. One of these lesions disappeared after a few days; the others became moist and a yellowish crust formed on each of them. All of these papules remained small and disappeared after three months, leaving tiny dark marks. The eruption was evidently very irritable, as the monkey was continually scratching. It is possible that the papules observed two months after the first lesion appeared, may represent results of auto-inoculation by scratching rather than a true secondary eruption.

*Monkey No. 17 (Semnopithecus priamus).* 15. x. 06. Scrapings taken from a non-ulcerated papule of a yaws patient were well rubbed into deep scarifications over the left eyebrow. Forty-five days later three slightly elevated spots appeared which soon fused together into an infiltrated mass, covered by a thick crust; the lesion increased to the size of a sixpenny piece. No other lesions appeared. The lesion is still present and of the same size (31. i. 1907). It was examined for the *Spirochaeta pertenuis* on three different occasions, twice with positive results.

Altogether eight monkeys of the genus *Macacus* and 11 of the genus *Semnopithecus* have been inoculated with scrapings taken from the eruptive lesions of yaws patients: the inoculation was successful in five monkeys of the first genus and nine of the second. The incubation period has varied from a minimum of 19 days to a maximum of 92. The appearance of the lesion developing at the seat of inoculation was practically the same in all cases, viz. an infiltrated spot slowly increasing in size and soon becoming moist, the secretion drying into a thick crust. When the crust was removed a raw granulating red surface was seen. With the exception of three cases the eruption remained localized at the point of inoculation and no other eruption appeared. Of the three cases in which eruption developed some time after the primary lesion, in one, as I have already mentioned (monkey No. 4), two small papules appeared on the lower part of the forehead, in the vicinity of the primary lesion, and two others above the upper lip. Of the other two monkeys, in one, a rather large moist papule appeared on the lower lip three months after the primary sore had developed; in the other, three small papules, which soon broke and became covered with a crust, developed on the lower part of the forehead, close to the primary lesion, two and a half months after the first lesion had appeared. See Plate XV.

*Inoculation of Monkeys with the Blood of the General Circulation  
from a Yaws Patient.*

About 5 c.c. of blood were withdrawn (19. ix. 1906) aseptically from a vein at the bend of the elbow of a patient suffering from a typical yaws eruption on the legs, back and face, but not on the arms ; the needle, therefore, could be inserted through perfectly normal skin without touching any yaws lesion. One c.c. of the blood was well rubbed into deeply scarified spots on the right eyebrow of a *Macacus*. Thirty-three days later, a small deeply raised, brownish papule appeared. Before the papule became moist a scraping was taken and stained with Leishman's method in the manner I have described elsewhere (26. xi. 1905). Many *Sp. pertenuis* were present. The papule slowly enlarged and became covered with a crust. The lesion disappeared after three months ; no other lesions developed.

This experiment shows that :

1. Monkeys can be successfully inoculated with the blood of a yaws patient.

2. The *Spirochaeta pertenuis* is, at least temporarily, present in the blood of the general circulation, though so far I have not been able to detect it microscopically.

*Inoculation of Monkeys with the Splenic Blood derived from a  
Case of Yaws.*

19. ix. 1906. About 1 c.c. of splenic blood was obtained by puncturing the spleen of a patient affected with typical yaws ; film preparations showed very rare *Sp. pertenuis*. The splenic blood was inoculated into two *Mac. pileatus* in the usual manner.

The result was positive in one monkey, a framboetic papule developing after an incubation period of 36 days ; the result was negative in the other monkey.

*Inoculation of Monkeys with the Cerebro-spinal Fluid of Yaws  
Patients.*

15. ix. 1906. Four monkeys (2 *Mac. pileatus* and 2 *Semn. priamus*) were inoculated with the cerebro-spinal fluid derived from three different patients affected with yaws. The cerebro-spinal fluid was in all the cases perfectly limpid ; on centrifugalization it did not show any sediment and *Sp. pertenuis* could not be found, though it was present in the skin lesions of the same patients.

Up to date (15. ii. 1907), four and a half months after inoculation, the result has been negative.

*Inoculation of Filtered Yaws Virus.*

14. IX. 06. The scrapings were taken from non-ulcerated human papules containing *Sp. pertenuis* in exceptional abundance. No other germs could be detected microscopically or by cultural methods. The scrapings were mixed and well triturated with normal saline solution. Preparations made from this mixture showed many *Sp. pertenuis*. Part of the mixture was then inoculated in the usual manner into two *Mac. pileatus*. The rest of the mixture was filtered through a Berkefeld filter (12 A); preparations made from the filtrate did not show the presence of the spirochaete. The filtrate was inoculated into three *Mac. pileatus* and one *Semn. priamus*. Both monkeys inoculated with the unfiltered material developed—one after 25 days, the other after 40 days—framboetic papules at the seat of inoculation, covered by a thick crust. Films made from the scrapings of the framboetic lesions of both monkeys contained *Sp. pertenuis*. The four monkeys inoculated with filtered material have not shown any lesions either at the place of inoculation or in any other region of the body, though six months have elapsed since the inoculation.

This experiment tends to prove that *Sp. pertenuis* is the true cause of yaws, for when it is removed from yaws material, the latter is no longer infective.

*Inoculation of Syphilis in Monkeys previously Inoculated with Yaws.*

*Monkey No. 4 (Mac. pileatus)* was successfully inoculated with yaws in February, 1906. On 16. VI. 1906, scrapings from a primary sore of a syphilitic man were well rubbed into scarified spots on the prepuce of the monkey's penis. On the 26th day after inoculation a small vesicle surrounded by a reddish halo appeared. The vesicle burst, leaving an erosion surrounded by infiltrated tissue. The glands of both groins became enlarged and hard, and could be easily felt. No secondary eruption appeared, but, as shown by Metchnikoff and Roux, this is almost always the case when experimenting with monkeys of a low type.

*Monkey No. 11 (Mac. cynomolgus)*, 21. VIII. 06. Inoculated with yaws material on the left eyebrow; a framboetic papule developed on 22. IX. 06. Inoculated over the right eyebrow with syphilitic virus taken from a primary human sore on 30. XI. 1906. After 42 days a brownish papule developed surrounded by an infiltrated zone.

The monkey still presents (15. II. 1907) the framboetic lesion as well as the syphilitic sore; no secondary eruption has appeared.

*Simultaneous Inoculation of Yaws and Syphilis.*

*Monkey No. 27 (Mac. pileatus)* 10. IX. 06. Inoculation (1) with human yaws virus taken from a non-ulcerated papule on the left eyebrow, and (2) human syphilitic virus on the right eyebrow. The syphilitic material was taken from a primary human sore. After 32 days the left eyebrow, inoculated with yaws, showed three small flattened papules, which fused together into an elevated infiltrated mass

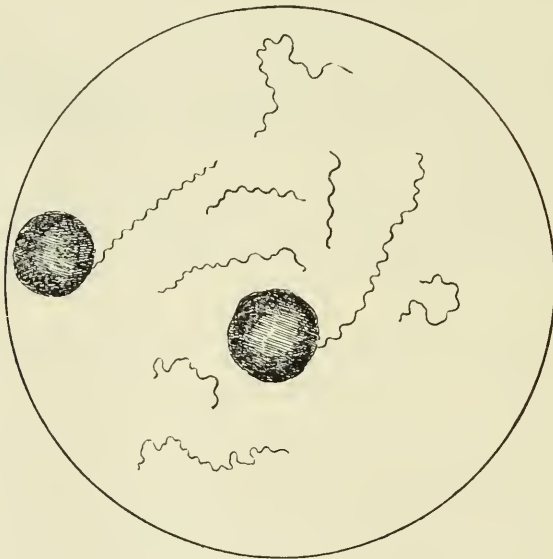
the size of a pea, covered by a thick crust. The right eyebrow, inoculated with syphilitic material, 39 days after inoculation, presented a tiny brown papule, which soon broke and became covered with a slight crust.

As regards the appearance of the yaws and syphilitic lesions, the yaws lesion was larger, more elevated and covered by a much thicker crust. The syphilitic lesion disappeared after two months, while the framboetic lesion is still present (15. II. 1907).

*Transmission of Yaws from Monkey to Monkey.*

*Monkey No. 21 (Mac. pileatus)* was inoculated on the left eyebrow with the virus of human yaws taken (19. IX. 06) from a non-ulcerated papule. From the infiltrated spot, which appeared 40 days later and which contained *Sp. pertenuis*, a scraping was taken and inoculated (22. XI. 06) into three *Mac. pileatus* and four *Semn. priamus*.

Of the three monkeys of the same species as No. 21, two gave positive results, the incubation period being 31 days in one case and 42 in the other. Of the four monkeys of a different species to No. 21, one only gave a positive result, after an incubation period of 67 days.



*S. pertenuis*. Preparation taken from a monkey inoculated with *Framboesia*.

*Incidence of the Sp. pertenuis<sup>1</sup> in Monkeys Inoculated with Yaws, in comparison with the incidence of the Sp. pertenuis in Man suffering from Yaws.*

The results of the investigation are collected in the following two tables.

TABLE I.

*Incidence of the Spirochaeta pertenuis in monkeys inoculated with yaws.*

Material investigated	No. of monkeys examined	No. of monkeys in which positive results were obtained
Primary lesion at the seat of inoculation ...	16	15
Framboetic papules which appeared some time after the primary lesion ...	3	2
Spleen juice ...	4	3
Bone marrow ...	4	1
Blood, general circulation ...	15	0
Smears from liver ...	4	0
Lymphatic glands ...	6	3
Brain substance ...	4	0
Cerebro-spinal fluid ...	4	0

TABLE II.

*Incidence of the Spirochaeta pertenuis in yaws patients.*

Material investigated	No. of cases examined	No. of cases in which positive results were obtained
Primary lesion ...	6	6
Unbroken papules of the general eruption ...	76	73
Ulcerated papules of the general eruption ...	76	52
Blood of the general circulation ...	20	0
Spleen blood ...	5	3
Cerebro-spinal fluid ...	6	0
Lymphatic glands ...	11	6

Comparing Table I with Table II, it will be seen that the incidence of *Sp. pertenuis* is practically constant in the eruptive lesions both in man and in inoculated monkeys. In the monkeys I have experimented with, the eruption does not become general, as in man; notwithstanding this we must admit that in monkeys also we have to do with a generalized

<sup>1</sup> This spirochaete was found by me in a case of yaws in February 1905, when I considered it to be a *Spirillum*. The discovery by Schaudinn of a spirochaete in syphilis, published soon afterwards, induced me to work at the subject in a systematic manner. The yaws spirochaete was first described by me (17. vi. 1905) under the name of *Spirochaeta pertenuis*. Later, (16. xi. 1905), I suggested the name of *Sp. pallidula*, on account of its resemblance to the spirochaete found in syphilis. According to the laws of nomenclature, however, the correct name is *Sp. pertenuis*.



infection, as proved by the presence of *Sp. pertenuis* in the spleen and lymphatic glands.

*Microscopic Examination of the Lesions in Experimental Yaws.*

*Monkey No. 4 (Mac. pileatus).* In this monkey, 19 days after inoculation, as already described, a small infiltrated spot appeared at the point of inoculation over the left eyebrow. The lesion became moist, the secretion drying into a thick crust and attaining the size of a sixpenny piece in about two weeks. Two months later, the first lesion being still present, of the same size and with the same characters, four more papules appeared; two close to the first lesion and two just above the upper lip. These papules remained always of small size and disappeared within three months. It is possible that these four papules were due to auto-inoculation by scratching; it cannot be excluded, however, that they might represent a partial secondary eruption, comparable to the general secondary eruption which appears in man; it must be remembered that though the skin lesions in experimental yaws—with the monkeys I have used—are generally localized at the point of inoculation, the infection is general, as clearly proved by the presence of the *Sp. pertenuis* in the spleen of the animals.

On 1. VI. 1906 the crust from the primary lesion was removed; from the raw, elevated, granulating surface, a piece of tissue was cut, divided into small portions and fixed in different ways (alcohol, sublimate, etc.); then imbedded in paraffin. Sections were stained by various methods (Pappenheim's, etc.). The two papules which appeared above the upper lip were also removed and investigated by the same methods. The results of the histological examination are briefly the following:—

I. *Primary lesion.*

(a) A well marked proliferation of the interpapillary processes.

(b) A cellular infiltration consisting of (1) numerous typical plasma cells, found diffusely, with no definite arrangement; (2) some extravasated polymorphonuclear leucocytes; (3) small mononuclear leucocytes, connective tissue cells and a few mast-cells. No true giant cells were observed. The fibrous stroma was very delicate and scarce.

II. *Papules removed from the lip* presented practically the same appearances, only the proliferation of the interpapillary processes is much less marked.

Comparing these results with those found by Macleod, Unna, Nicholls, and myself, in man, it would seem that the histological structure is practically the same in human as in experimental yaws.

*The Bordet-Gengou Reaction in Yaws.*

I have applied this reaction to yaws, following the technique used in syphilis by Wassermann, Neisser and Brück (10. v. 1906). As is well known, the principle of the reaction is as follows: when complement is mixed with the complex antigen + immune-body, and afterwards some sensitized red blood corpuscles are added, no haemolysis takes place, as the complement has been already taken up by the complex antigen + immune-body, and cannot, therefore, become fixed to the haemolytic receptors.

If the complex antigen + immune-body is absent, or only antigen or only immune-body is present, then the complement will remain free and on addition of the sensitized red blood corpuscles, will become fixed to the haemolytic receptors, and haemolysis will take place. From the absence or presence of haemolysis we can therefore detect the presence or absence of the complex antigen + immune-body. As the following experiments prove, it is possible to demonstrate the existence of specific yaws antibodies and antigen.

*Experiment I.* To the extract of non-ulcerated yaws papules containing abundant *Sp. pertenuis*, some serum (heated to 55° C.) was added, derived from a monkey which had been successfully inoculated with yaws and which had been afterwards treated at intervals with subcutaneous inoculations of yaws material. Then some fresh guinea-pig serum was added (complement) and, after a certain time, some sensitized red blood corpuscles—in my experiments goats' corpuscles—treated with inactivated serum from a rabbit which had been inoculated several times with goats' corpuscles.

Result: no haemolysis.

The experiment was repeated, using the extract of papules taken from six other cases of yaws. The result was constantly the same, namely, no haemolysis took place.

*Experiment II.* Same procedure as in Experiment I, using, instead of the extract of yaws papules, the extract of leprosy nodules.

Result: well marked haemolysis.

*Experiment III.* Same procedure, using the extract of nodules taken from a case of pseudo-granuloma pyogenicum.

Result: haemolysis.

*Experiment IV.* Same procedure, using, instead of the extract of yaws papules, the extract of syphilitic condylomata.

Result: haemolysis.

*Experiment V.* Same procedure, using the extract of a syphilitic primary sore which contained many *Sp. pallida* Schaudinn.

Result: haemolysis.

*Experiment VI.* Extract of yaws papules containing *Sp. pertenuis* + serum (heated to 55° C.) of a monkey immunized for syphilis + fresh guinea-pig serum + sensitized corpuscles.

Result : haemolysis.

The experiment was repeated, using the extract of papules from six different cases of yaws ; haemolysis always resulted. It is to be noted that the serum of the monkey contained, with certainty, syphilitic antibodies, as no haemolysis took place when it was inactivated and then had added to it the extract of a primary syphilitic sore, then fresh guinea-pig serum (complement), then sensitized corpuscles.

*Experiment VII.* Extract of yaws papules + serum (heated to 55° C.) derived from a normal monkey + fresh guinea-pig serum (complement) + sensitized corpuscles.

Result : haemolysis well marked.

*Experiment VIII.* Extract of spleen juice obtained by puncture of a case of typical yaws + inactivated serum of a monkey immunized for yaws + complement + sensitized corpuscles.

Result : no haemolysis.

*Experiment IX.* Same procedure as in Experiment VIII, using, instead of the serum of a monkey immunized for yaws, the serum of a monkey immunized for syphilis.

Result : haemolysis.

The above experiments show that it is possible to detect specific yaws antigen in the yaws papules and in the spleen of cases of yaws, and specific yaws antibodies in the blood of monkeys treated with inoculation of yaws material.

The experiments IV, V, VI, IX show also that yaws antibodies and antigen are different from syphilis antibodies and antigen, and therefore syphilis and yaws differ specifically.

*Communicability of Yaws. Do Insects play a part in the transmission of the Disease ?*

It is well known that yaws is in most cases conveyed by direct contact from person to person, usually by absorption of the virus through some pre-existing abraded surface, or through small wounds or ulcerations, which frequently are present on the skin of natives. The simple contact of the virus on normal skin is not sufficient to cause infection ; but very slight abrasions, for instance those due to scratching, are sufficient for the entrance of the virus.

Women are frequently infected by their children, the primary lesion appearing often on the mammae. In the native women of Ceylon the primary lesion frequently develops on the skin of the trunk just above



Fig. 1.

Fig. 1. Photograph showing how Ceylon women carry their children, the primary framboetic sore being often found in such women above the hip.



Fig. 2.

Fig. 2. Primary framboetic sore surrounded by lesions of secondary eruption. The same woman as in the picture alongside.





the hip (Plate XVI, Fig. 2). This is due to their habit of carrying the child astride of the hip, as shown on Plate XVI, Fig. 1. If, therefore, a yaws lesion is present on the scrotum or nates of the child it will be continually rubbed against the skin of the mother and she will become infected through any slight abrasion already present or through any abrasion which may be caused by the friction.

In my opinion, however, there can be little doubt that in certain cases insects may carry the disease. It is very noticeable that flies eagerly crowd on the open sores of yaws patients. In the hospitals, as soon as the dressings are removed, the yaws ulcerations become covered with flies sucking with avidity the secretion, which they may afterwards deposit in the same way on ordinary ulcers of other people. Ants are also occasionally found on yaws ulcerations as well as on ordinary ulcers.

In the classical work of Nuttall (1899, p. 34) on the rôle of insects as carriers of parasitic diseases, several authors are quoted (Alibert, Hirsch, Cadet, Wilson) who believe that the infection may be conveyed from one individual to another by flies. Wilson states that this belief prevails also among the natives of the West Indies.

I may quote some of the experiments I have made to prove that flies are instrumental in the dissemination of the disease.

*Experiment I* (10. XI. 06). Some scrapings were collected from slightly ulcerated papules of a yaws patient. The *Sp. pertenuis* was present, together with various other thicker spirochaetes. (*Sp. obtusa*; *Sp. acuminata*) but no bacteria. The scrapings were placed in a sterile Petri dish. Ten flies (*Musca domestica* and allied species), caught in the rooms of the Bacteriological Institute, were placed inside the Petri dish and left there for half an hour. They fed greedily on the material; then their mouth parts and legs were examined for spirochaetes, extracts and films being made: in nine flies the spirochaetes of the thicker types were found; in two also the *Sp. pertenuis*. As a control five flies were caught the same day, in the same room and examined at once, with negative results as regards the presence of spirochaetes.

*Experiment II* (12. I. 07). Twenty flies were collected from the rooms of the Bacteriological Institute. The buccal apparatus and legs of five were removed and examined by making extracts and films: no spirochaetes of any kind were present. The other 15 flies were divided in several groups and placed on various semi-ulcerated papules of three yaws patients presenting the *Sp. pertenuis*, and spirochaetes of the thicker type which are often found in semi-ulcerated lesions. The flies were kept in place by covering the papules with a piece of gauze made to adhere to the skin by means of collodion all round the margin. All the flies fed greedily on the ulcerated papules. After two hours the mouth parts were removed, extracts and films made and stained. Out of the 15 flies so examined, in 14 it was possible to detect the coarse spirochaetes, and in two, the *Sp. pertenuis*, as well as the thicker ones.

*Transmission of Yaws to Monkeys by means of Flies fed on Yaws Material.*

*Experiment III* (15. XI. 06). Thirty flies were fed in a sterile Petri dish for half an hour on scrapings taken from non-ulcerated papules of a case of yaws, containing only the *Sp. pertenuis*. Three *Semnopithecus priamus* and two *Macacus pileatus* were then infected in this way: over the left eyebrow of each monkey very numerous deep scarifications were made; then five flies, deprived of their wings, were applied to the scarified spots and kept there by means of a piece of gauze smeared with collodion at the margins; the monkeys were prevented from removing the gauze by tying their legs. After two hours the gauze and the flies were removed. Of these monkeys, one *Semnop. priamus* after 45 days developed a small infiltrated spot, which soon became enlarged and covered with a thick crust. The microscopical examination of the lesion showed the presence of *Sp. pertenuis*. The other five monkeys gave negative results.

*Experiment IV*. Twenty-eight flies (*Musca domestica* and similar species) were caught in one of the rooms of the Bacteriological Institute. The legs and buccal organs of five were removed and examined for spirochaetes, numerous preparations being made, with negative results. The remaining flies, deprived of their wings, were placed on two slightly ulcerated lesions of a yaws patient. The flies were kept on the ulcers by means of pieces of gauze, the margins of which were made to adhere to the skin with a little collodion. The flies readily sucked the secretion of the ulcers. After one hour the flies were removed. Meanwhile seven *Semnopithecus priamus* had been deeply scarified over their eyebrows, and several flies which had fed on the ulcerated yaws lesions were placed on the scarified areas of each monkey and kept in place there for two hours by using the device already described.

One of the monkeys, 46 days later, developed a slightly infiltrated spot, which slowly enlarged into a framboetic nodule covered by a thick crust; the microscopical examination of films taken from this nodule showed the presence of *Sp. pertenuis*. In another monkey, 67 days after inoculation, three tiny papules developed at the place of inoculation; they soon fused together into an infiltrated mass covered by a thick crust. Films made from scrapings of the lesion contained the *Sp. pertenuis*. The remaining five monkeys, so far (15. II. 1907), have given negative results.

SUMMARY AND CONCLUSIONS.

1. Monkeys are susceptible to yaws. The skin eruption in the monkeys I have experimented with (*Semnopithecus priamus* and *Macacus pileatus*) is, as a rule, confined to the seat of inoculation but the infection is general, as is proved by the presence of the *Spirochaeta pertenuis* in the spleen and lymphatic glands.

2. Material obtained from persons suffering from yaws and apparently containing *Spirochaeta pertenuis* only is infective to monkeys.
3. When the *Spirochaeta pertenuis* has been removed from this material by filtration, the latter becomes inert.
4. The inoculation of blood from the general circulation and blood taken from the spleen of yaws patients into monkeys may give positive results.
5. The inoculation of the cerebro-spinal fluid of yaws patients gives negative results.
6. Monkeys successfully inoculated with yaws do not become immune for syphilis.
7. Monkeys successfully inoculated with syphilis do not become immune for yaws.
8. By means of the Bordet-Gengou reaction, it is possible to detect specific yaws antibodies and antigen.
9. Yaws antibodies and antigen are entirely different from syphilitic antibodies and antigen.
10. The presence of the *Spirochaeta pertenuis* in monkeys experimentally inoculated, as well as in yaws patients, is practically constant in the unbroken eruptive lesions; the *Spirochaeta* is frequently present in the spleen and lymphatic glands.
11. Yaws is generally conveyed by actual contact, but under certain circumstances it may be conveyed by flies and possibly by other insects.

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## SOME EXPERIMENTS ON THE FILTRATION OF CATTLE PLAGUE BLOOD.

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(Seven charts.)

THE outbreak of cattle plague in Egypt and the establishment of the Serum Institute at Abbassieh offered a suitable opportunity for investigating some points concerning the nature of the causal agent of the disease, and especially the important question whether the virus is, or is not, capable of traversing a filter candle. On this point the literature is strangely contradictory.

Semmer (*Deutsche Zeitschr. f. Tiermed. u. vergl. Path.*, 1896, vol. XXII. p. 32) found that infective materials after passage through a Chamberland filter were non-virulent.

Nencki, Sieber and Wyznikiewicz (*Centralbl. f. Bakt.*, 1898, Abt. I, vol. XXIII. p. 535) found that the filtrates from both Berkefeld and Chamberland filters were non-virulent.

Kolle and Turner (*Zeitschr. f. Hyg.*, 1898, vol. XXVIII. p. 361) state that the microbe does not pass through Pasteur, Chamberland or Berkefeld filters, and that when virulent blood is passed either slowly or quickly through such filters the filtrate, even in large quantities, is not infectious, whilst the material remaining on the filters is highly so.

To eliminate the possibility of this result being caused by the microbe being an obligatory intracorpuseular parasite and so never being really free in the blood, the corpuscles were haemolysed by the addition of 0.2% sodium chloride solution, and it was found that this had no influence on the result.

Kolle and Turner are of opinion that the parasite is not so small as to be invisible to modern lenses, but at the same time hold that the probability of seeing it is very small as it must be at any rate more minute than an influenza bacillus.



Kolle (*Zeitschr. f. Hyg.*, 1899, vol. xxx. p. 36) showed that it defibrinated cattle plague blood—either haemolysed or not—be centrifuged at a speed of from 2900 to 3000 revolutions per minute for 20 to 30 minutes, the parasite is entirely driven down with the deposit, which is highly infectious, the supernatant fluid being quite free from infection.

Nicolle and Adil-Bey in their first paper (*Ann. Inst. Pasteur*, 1899, vol. xiii. p. 323), agree completely with the above results; they say: "Si l'on filtre le sang, defibriné et étendu au dixième, sur le filtre Chamberland ou sur la bougie Berkefeld, le liquide se montre inoffensif, mais il ne vaccine pas," but in a subsequent paper (*ibid.*, 1902, vol. xvi. p. 56) the authors state that the virus is capable of traversing Berkefeld and also, with greater difficulty, Chamberland F candles, but only under certain conditions. They conclude that the microbe is commonly intraleucoeytic.

Yersin (*ibid.*, 1904, vol. xviii. p. 429), working with the fluid obtained by washing the peritoneum in animals suffering from the disease (he injected salt solution intraperitoneally and removed it after four hours), found that the virus passed through Chamberland filters (mark F) but was stopped by mark (B).

Memmo, Martoglio and Adani (*Ann. d' Ig. Sper.*, 1904, vol. xiv. p. 256), working in Eritrea, come to the conclusion that the virus passes the Berkefeld candle, but is stopped by the Chamberland filter.

There is thus a very wide discrepancy in the results of the different workers. Semmer, Nencki, Sieber and Wyznikiewicz, and Kolle and Turner all agree that the virus does not pass through a filter, and the last-named authors find that it is not only incapable of passing through an ordinary Berkefeld filter, but is comparatively easily removed from suspension by centrifugalisation.

Memmo, Martoglio and Adani, on the other hand, find that it passes the Berkefeld candle but not the Chamberland filter, while Nicolle and Yersin find that it passes a Chamberland filter.

Filtration experiments, in the case of a disease so highly infectious as cattle plague, are surrounded by many possibilities of error. Apart from the question of a flaw or other weakness in the filter candle itself, which can be more or less easily controlled, there is the great danger of accidental infection of the inoculated animals. Unless the strictest precautions are taken this may take place through channels which would hardly be suspected by persons who had not had an actual acquaintance with the disease.



For this reason one is inclined to place much more reliance on a few negative results, carried out under strict conditions, than on a larger number of experiments giving positive results.

Nicolle and Yersin, who both found that the virus passes a Chamberland filter, are by no means in agreement as to the ease with which this takes place. Nicolle says:—"On réussit *rarement* et encore n'arrive-t-on qu'à vacciner les animaux," whilst Yersin finds that "Le virus traverse *constamment* la bougie F." The latter author unfortunately only quotes one experiment, and in this does not give the temperature chart of the animal inoculated, so that it is impossible to form an opinion. Nicolle gives complete details of three animals inoculated with Berkefeld filtrates—two of these remained well but were afterwards found to be immune, the third contracting a fatal attack of the disease after a long incubation period. The initial rise of temperature took place on the morning of the eighth day, and Nicolle attributes this long incubation period to the small number of germs inoculated and to their dilution. This explanation is, however, somewhat difficult to accept, and is certainly not in agreement with the experience at Abbassieh, where it was found that with very small doses of virulent blood (down to  $\frac{1}{500}$  c.c.) the temperature rose after exactly the same incubation period as in the case of larger doses.

This constancy of the incubation period is most definite, at any rate in Cyprus cattle, and on comparing the temperature charts of a large number of cases of the experimentally produced disease one is struck by the remarkable similarity of the temperature curves. The most characteristic point is the almost constant incubation period before the initial rise of temperature. These facts are well shown in the annexed charts.

To eliminate small accidental variations the temperature charts of groups of four consecutive animals (without any selection) were taken. Beginning with the first day, the morning temperatures of each of the four animals were added together and divided by four, thus giving the average temperature of the four animals for that morning. This was repeated for the morning and evening observations of six days and the average temperature charted on one chart, which was the average temperature chart for four animals.

Chart 1 (*a, b, c, d*) shows a group of four of these charts, each representing the average of a group of four animals. The similarity is very striking.

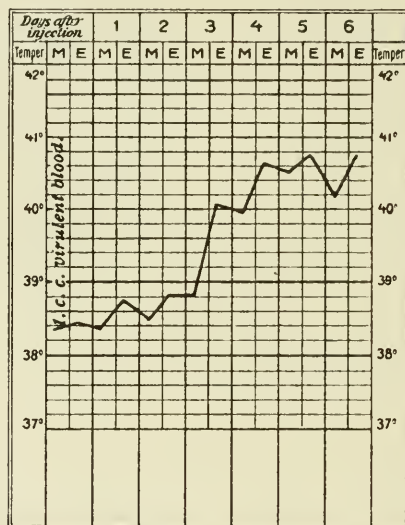
Chart 2 is a chart similarly obtained by averaging the temperature



charts of a series of 20 consecutive Cyprus animals, and may be taken as the typical chart of the experimentally produced disease in the Cyprus animal.

From this it will be seen that the first rise of temperature takes place with great regularity on the evening of the third day, *i.e.* 72 hours after the inoculation.

CHART 2.



To further establish this a series of 637 charts of experimentally infected Cyprus animals was examined, and a note made of the exact period at which the first rise of temperature occurred. The results are as follows:—

Rise of temperature	No. of animals	Per cent. (approx.)
After 1 day	0	0
„ 2 days	97	15
„ 3 „	437	69
„ 4 „	94	14.5
„ 5 „	9	1.5
„ 6 „	0	0

The injections of virulent blood were unfortunately not made at the same hour every day during the period under observation, but varied from 9 a.m. to 6 p.m. But for this fact the results would probably have been even more striking. The few cases in which the rise did not occur

until after five days were probably largely accounted for by some accidental circumstance, *e.g.* the thermometer not being left sufficiently long in the rectum; moreover in winter the Cyprus stables were very much exposed to the cold south wind, and, as the animals were usually in very poor condition, they not unfrequently showed a subnormal temperature.

This typical temperature curve is most valuable in the diagnosis of the disease, and in cases where an experimental inoculation is made for diagnostic purposes it is the temperature chart as a rule which gives the most useful evidence. That this is the case in other parts of the world is shown by Yersin's statement with regard to the epizootic in Indo-China; he says (*loc. cit.*):—

“Les seuls symptômes constants, que nous avons presque toujours observés chez les animaux infectés par nous, ont été la durée de l'incubation, la période d'hyperthermie sans rémission et la diarrhée.”

From the above it is seen that out of 637 animals inoculated with virulent blood in no case did the initial rise of temperature occur later than five days after inoculation. One is therefore inclined to regard the animal referred to by Nicolle as having contracted the disease accidentally at some date subsequent to the injection of the filtrate.

This accidental infection is by no means easy to avoid, as was realised in the first filtration experiment made at Abbassieh.

In this case two Cyprus bulls were inoculated with 200 c.c. of the filtrate of virulent blood diluted to 1 in 4 with saline solution before filtration. The animals were placed outside the compound in a separate stable and ordinary precautions were taken to avoid infection, but they were attended to by members of the staff of the Institute. The temperatures of both animals began to rise on the fifth day after the injection, and both animals developed typical cattle plague. As it is exceedingly rarely if ever that the incubation period in Cyprus animals lasts so long as this after subcutaneous inoculation, while after infection by the mucous membranes (*e.g.* smearing the nostrils with infective materials) this period is the rule, it was almost certain that these animals had in some way become infected naturally, probably during handling in the course of injection. Further experiments were therefore postponed until they could be done under thoroughly strict conditions.

A very striking point in Nicolle's results is the number of cases in which he obtained “vaccinating filtrates,” *i.e.* the animal remained well after the injection of the filtrate, and when tested with virulent blood,

from 10 to 15 days later, resisted infection. This in itself is a most interesting fact, and it is a pity that the temperature charts are not given.

It would be interesting to know if the animals employed (animals from Anatolia, Crimea, etc.) are not sometimes immune.

In an earlier paper Nicolle states that he has tested the comparative susceptibility of the various races most commonly found in Turkey, and that the Crimean, Anatolian and Egyptian animals are very susceptible, and that in them death constantly takes place after the injection of virulent blood. For the Egyptian animals, in Egypt at any rate, we know that this is by no means the case.

In repeating these experiments on the filtration of virulent blood the greatest precautions were taken to obviate all possible chances of accidental infection. The filtrates were tested on cattle imported directly from Cyprus. These Cyprus cattle were very highly susceptible to cattle plague—out of 1098 inoculated with virulent blood in the course of the routine work of the Institute only one proved to be immune; this was a very old cow, whose previous history was of course unknown.

The Cyprus cattle which were used remained in the stables where the non-infected Cyprus cattle for the Institute were kept, and so were together with a considerable number of clean Cyprus animals, which served as controls. These stables were about a mile from the Institute and had an entirely different staff, which had no connection with that of the Institute.

Virulent blood for the experiment was defibrinated by whipping, diluted with four times its volume of 0·8% salt solution and divided into two parts. One part (*a*) was passed through a large Berkefeld filter, which was somewhat close-pored, and the filtration was done as slowly as possible. The other part (*b*) was filtered as rapidly as possible through a large and very porous Berkefeld filter, and in this case ran through very rapidly. Filtration was carried out by suction from an ordinary laboratory water-pump, and the difference in pressure was therefore less than 1 atmosphere. In both cases a culture of an exceedingly small bacillus was mixed with the blood before filtration, but the bacillus did not pass the filter.

50 c.c. of each of the filtrates were injected subcutaneously into two Cyprus animals, care being taken that no one who had anything to do with cattle plague should come in contact with the animals. The temperature of all the four cattle remained normal—except one, which had a mild attack of red-water—until 10 days later, when they were



each inoculated with 10 c.c. of virulent blood. In each case the animals developed typical cattle plague, the initial rise of temperature occurring in two animals on the third day and in the other two on the fourth day. All the four animals were bled to death for virulent blood, and the diagnosis of cattle plague verified by post-mortem examination.

Three control animals inoculated with  $\frac{1}{5}$  c.c. of the virulent blood before it was filtered developed the disease typically, the initial rise of temperature occurring in two of the animals on the third and in the other on the fourth day.

The temperature charts of the four cattle which received the filtrate are given (Charts 3, 4, 5 and 6) on pp. 578, 579.

This experiment shows that highly virulent cattle plague blood, when diluted to five times its volume with saline solution, is rendered absolutely non-virulent even by rapid filtration through a porous Berkefeld candle. This being the case, it appears obvious that the microbe must be unable to traverse the much closer grained Chamberland candle, but it was thought interesting to try an experiment to establish this.

With this idea about 40 c.c. of citrated virulent blood was introduced by means of a pipette into the interior of a sterilised Chamberland filter F. The greatest care was taken to keep the filter aseptic and to avoid touching the neck with the virulent blood. A sterile cork was then placed in the neck of the candle, which was finally sealed with sealing-wax. The filter containing the virulent blood was then introduced with all surgical precautions into the peritoneal cavity of a Cyprus animal through a small incision in the abdominal wall, which was afterwards very carefully and thoroughly closed with sutures.

The operation succeeded well, and was followed by practically no rise of temperature. The wound healed by first intention, and the animal remained perfectly well for 13 days, when it was tested by an injection of virulent blood. This gave rise to a typical attack of the disease, death occurring eight days after the injection of virulent blood, showing that not only did the virus fail to pass the filter but that no immunity whatever resulted.

The chart of this animal is given (Chart 7) on p. 580.

Reviewing the work which has been published on this question and the results of the experiments cited above, one is led to the conclusion that there is insufficient evidence in favour of the active agent of cattle plague being capable of passing through a filter.

## FILTRATION EXPERIMENTS.

CHART 3.

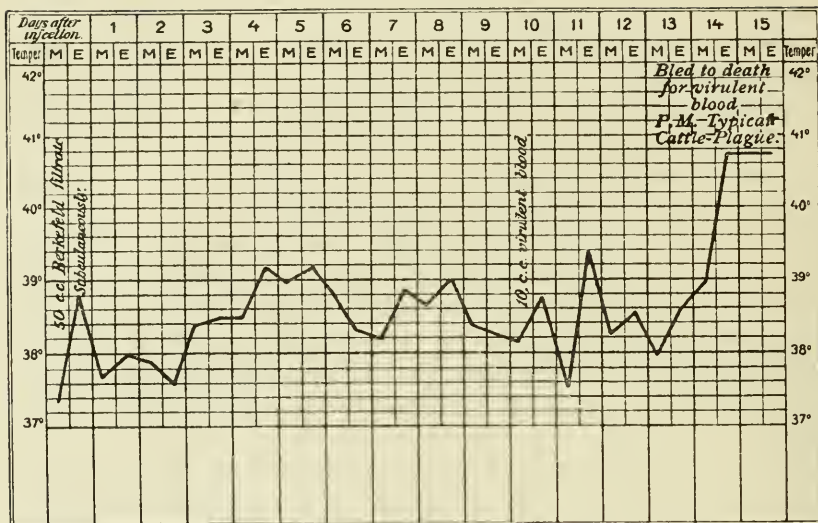


CHART 4.

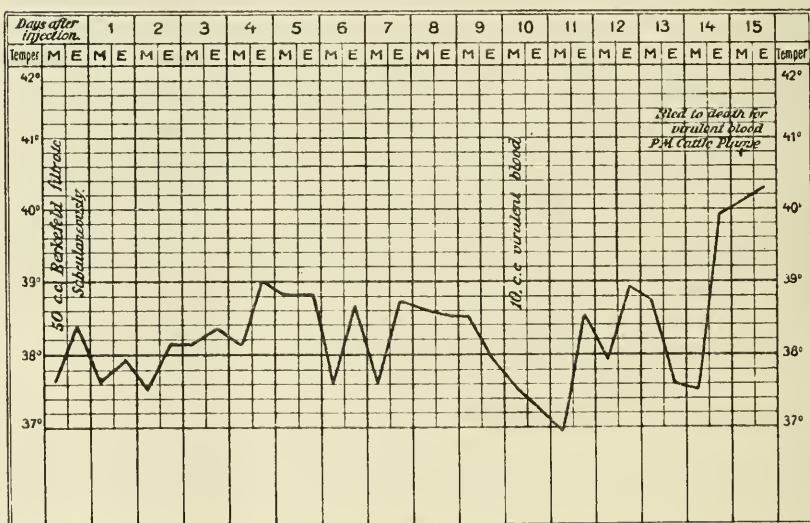


CHART 5.

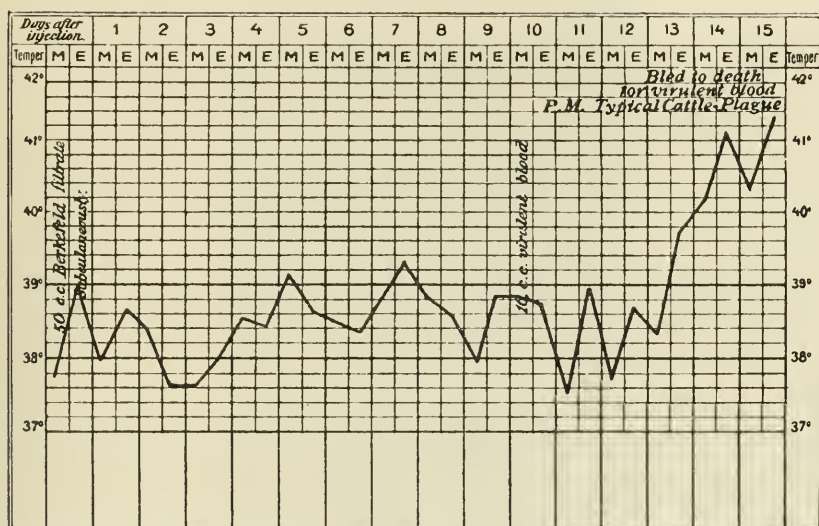


CHART 6.

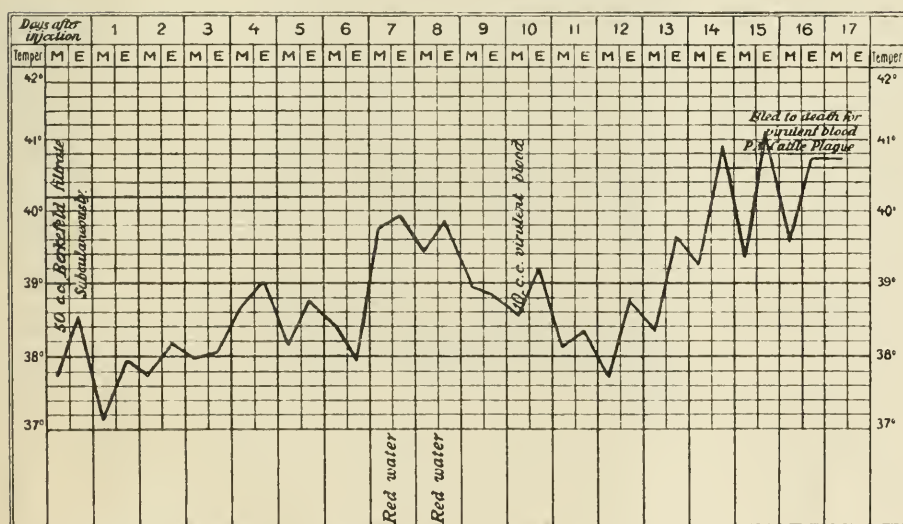
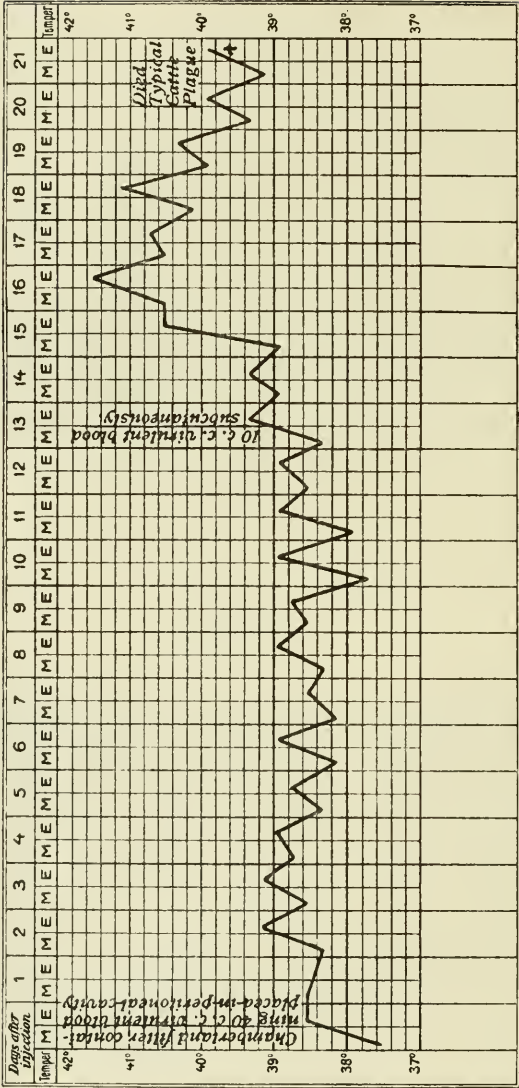


CHART 7.



## THE PARA-DIMETHYL-AMIDO-BENZALDEHYDE TEST FOR INDOLE<sup>1</sup>.

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(Three Charts.)

THIS indole reaction, first described by Ehrlich, and used by him as a urinary test, has been applied by Böhme in determining the presence of indole in bacterial cultures. Böhme claims for this test that it is more delicate and more exact than the usual nitrite and sulphuric acid test, and it has given such good and constant results in this laboratory, that the following facts and figures may be given in confirmation of Böhme's claim.

The test, as recommended by Böhme, consists of two solutions made up as follows :—

Solution 1.	Para-dimethyl-amido-benzaldehyde	...	4 parts.
	Absolute Alcohol	... ..	380 „
	Concentrated Hydrochloric Acid	... ..	80 „

Solution 2. Potassium persulphate in saturated watery solution. To about 10 c.c. of the broth culture of the organism add 5 c.c. of solution 1, and then 5 c.c. of solution 2, shake the mixture and the presence of indole is indicated by the appearance, in a very short time, of a red colour, which gradually becomes darker on standing

This test can not only be used qualitatively for detecting the presence of indole, but it can also be used quantitatively for estimating the amount of indole produced by the micro-organism.

*As a Qualitative test.* I think most observers will agree that the nitrite and sulphuric acid test is not always satisfactory, and there is often great doubt as to whether a slight reaction really means the

<sup>1</sup> The usual spelling "indol" is here altered to "indole" in accordance with a rule laid down by the Chemical Society whereby the termination "ol" is in future to be used to indicate substances containing an OH group.—ED.



production of indole by the micro-organism. The formation of indole, however, can be accurately determined by distillation and this fact was used as a confirmation in comparing the two tests, which, for the sake of brevity, we will now call the "old" (nitrite and sulphuric acid) and the "new" (para-dimethyl-amido-benzaldehyde).

Fifteen different micro-organisms were inoculated into peptone beef broth, and, at various intervals of time, were tested for indole, both tests being used. The results obtained are shown in the following table:

Micro-organism	24 hours' culture		3 days' culture		5 days' culture		12 days' culture	
	New test	Old test	New test	Old test	New test	Old test	New test	Old test
<i>B. enteritidis</i> (Gaertner)	0	0	0	+	0	+	0	trace
<i>B. typhi</i> murium	0	trace	0	+	0	trace	0	+
<i>B. psittacosis</i>	0	trace	0	+	0	+	0	trace
<i>B. Hanstedt</i> (Fisher)	0	0	0	+	0	+	0	+
<i>B. cloacae</i>	0	+	0	+	0	+	0	+
<i>B. of epidemic jaundice</i>	+	+	+	+	+	+	+	+
<i>B. acidi lactici</i>	+	+	+	+	+	+	+	+
<i>B. Hog cholera</i>	0	trace	0	+	0	+	0	trace
<i>B. Abel</i>	0	+	0	+	0	+	0	+
<i>B. coli communis</i>	+	+	+	+	+	+	+	+
<i>B. dysenteriae</i> (Flexner-Gray)	+	trace	+	+	+	+	+	+
<i>B. pyogenes fetidus</i>	+	trace	+	+	+	+	+	+
<i>B. typhosus</i>	0	trace	0	trace	0	+	0	+
<i>B. paratyphoid A</i>	0	trace	0	+	0	+	0	+
<i>B. paratyphoid B</i>	0	trace	0	+	0	+	0	+

According to the new test only five micro-organisms in the above series produced indole, namely, *B. of epidemic jaundice*, *B. acidi lactici*, *B. coli*, *B. dysenteriae* (Flexner-Gray) and *B. pyogenes fetidus*. Accordingly, large quantities of peptone beef broth, 50 c.c. to 100 c.c., were inoculated with these micro-organisms, incubated at 37°C. for some days, and then distilled. The distillate was tested for indole as before.

Micro-organism	Amount of culture	Time grown at 37° C.	Distillate	
			New test	Old test
<i>B. coli communis</i>	100 c.c.	9 days	+	+
<i>B. dysenteriae</i> (Flexner-Gray)	100	8	+	+
<i>B. pyogenes fetidus</i>	50	9	+	+
<i>B. acidi lactici</i>	50	9	+	+
<i>B. of epidemic jaundice</i>	100	5	+	+

This proved that these five micro-organisms, which always gave a constant reaction with the new test, were real indole producers, and the question next arose as to whether the positive reaction given with the old test by the other micro-organisms denoted the presence of indole. The following were therefore also distilled and the distillate tested.

Micro-organism	Amount of culture	Time grown at 37° C.	Distillate	
			New test	Old test
<i>B. enteritidis</i> (Gaertner)	100 c.c.	8 days	0	0
<i>B. Abel</i>	100	8	0	0
<i>B. cloacae</i>	100	6	0	0
<i>B. paratyphoid A</i>	100	7	0	0
<i>B. paratyphoid B</i>	100	7	0	0
<i>B. typhosus</i>	100	8	0	0

When, in addition to these results, we find that the para-dimethyl-amido-benzaldehyde gives a definite pink colour with '0001 milligramme of indole, whereas the nitrite and sulphuric acid test is barely perceptible with '0005 milligramme of the substance and fails entirely with '0002 milligramme, we must conclude that the para-dimethyl-amido-benzaldehyde test is both more accurate and more sensitive.

*As a Quantitative test.* Herter and Foster, and Peckham, have described methods for the quantitative determination of indole, the former observers using *B. naphthaquinone-sodium-monosulphonate* and the latter sulphuric acid and nitrite. I have endeavoured to ascertain whether para-dimethyl-amido-benzaldehyde can also be used colorimetrically in the quantitative estimation of indole, and have found the following method the most satisfactory.

A standard solution of indole is required, and is obtained by dissolving '05 gramme of indole in 5 c.c. of absolute alcohol and then adding distilled water to 500 c.c. Five c.c. of the distillate to be tested for indole are then taken (having added 1 c.c. of absolute alcohol for every 100 c.c. of the distillate), added to a Nessler's tube and made up to 50 c.c. with distilled water. To this add 2 c.c. of the para-dimethyl-amido-benzaldehyde solution and 2 c.c. of the potassium persulphate solution and thoroughly mix. As before, the presence of indole is indicated by the appearance of a red colour. Varying quantities of the standard solution are then similarly treated in a series of Nessler's tubes until the requisite colour is obtained, when the amount of indole in the distillate can be calculated. The mixtures should be allowed to stand for one hour before matching the colours, as in some tubes the colour sometimes develops more quickly than in others in which there is more indole and which eventually gives a darker colour. A series of Nessler's tubes, containing, say, '2, '3, '4 and '5 c.c. of the standard solution of indole give quite distinct grades of colour, so that in two solutions, a difference of '01 milligramme of indole can be accurately determined.

In testing this method the following three series of experiments were done, in order to show the variations in the amount of indole, produced by the same micro-organism, grown for different periods of time.

Seven flasks, each containing 100 c.c. of peptone beef broth were inoculated with 1 loopful of an agar culture of *Bacillus coli*, incubated

at 37° C. and distilled on succeeding days. The distillates, tested for indole, gave the following results:

Distillate after 1 days' growth = 0.86 milligrammes of indole per 100 c.c.

"	"	2	"	"	= 1.20	"	"	"	"
"	"	3	"	"	= 1.80	"	"	"	"
"	"	4	"	"	= 2.00	"	"	"	"
"	"	5	"	"	= 3.80	"	"	"	"
"	"	6	"	"	= 3.60	"	"	"	"
"	"	7	"	"	= 2.40	"	"	"	"

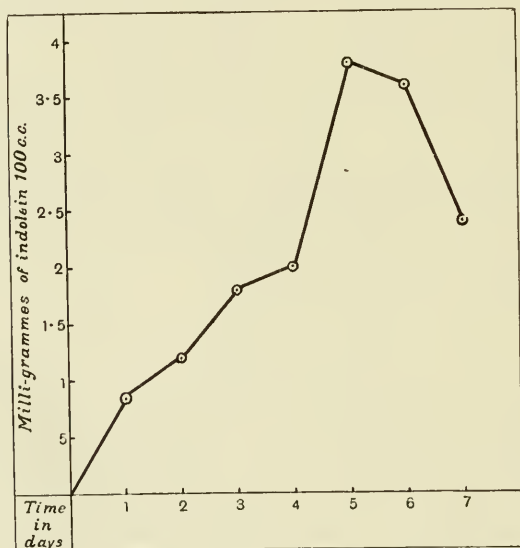


CHART I. Showing the amount of indole present on 7 consecutive days during the cultivation of *Bacillus coli* at 37° C.

In this series the indole production gradually rose to a maximum on the fifth day, with a slight falling off on the sixth day, and a still greater falling off on the seventh day. It was considered advisable to repeat this experiment with *Bacillus coli*, but to carry it on for a longer time. Another series of 100 c.c. flasks of peptone beef broth were inoculated with 5 drops, with a sterilized pipette, from a 24 hours' broth culture of *Bacillus coli*, this being considered more accurate than the standard loop. As before these flasks were incubated at 37° C., the first distillation carried out after four days' growth and the last 23 days after inoculation. The distillates gave the following figures:

Distillate after 4 days' growth = 1.20 milligrammes of indole per 100 c.c.

"	"	5	"	"	=1.30	"	"	"	"
"	"	6	"	"	=1.80	"	"	"	"
"	"	7	"	"	=1.60	"	"	"	"
"	"	10	"	"	=1.80	"	"	"	"
"	"	11	"	"	=2.00	"	"	"	"
"	"	14	"	"	=2.90	"	"	"	"
"	"	23	"	"	=1.80	"	"	"	"

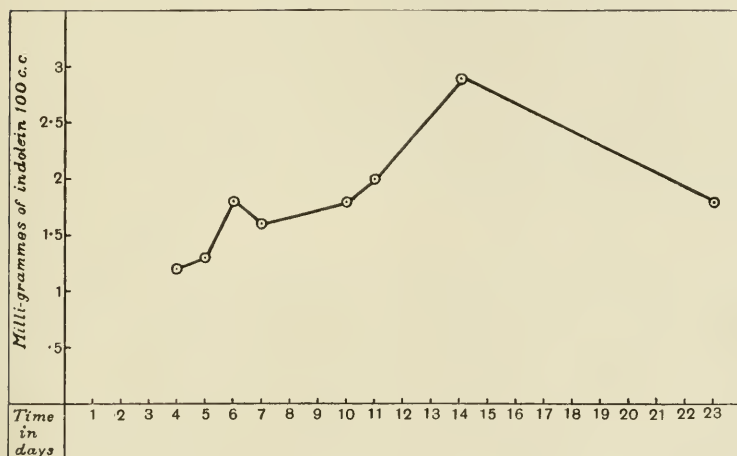


CHART II. Showing the amount of indole present at various stages during the cultivation of *Bacillus coli* for 23 days at 37° C.

In this series the indole production was very much slower, probably on account of the different method of inoculating the flasks. The indole production rose from the fourth to the sixth day, fell a little on the seventh and then rose to a maximum on the fourteenth day.

It was then considered advisable to estimate the indole production by another micro-organism, and cholera was chosen, because it was found on estimation to produce more indole than any other of the indole-producing organisms we had tested. This choice was rather unfortunate, as the nitrite produced by the cholera vibrio was sometimes present in the distillate and it was found that the presence of a nitrite has an inhibitory action on the production of the red colour. Nitrites in strong solutions give a yellow colour on the addition of the reagents. In this series, as in the preceding one, five drops of a bouillon culture were used to inoculate the flasks. The first distillation on this occasion, was done early, seven hours after inoculation, and the last flask had been

incubating 27 days before it was distilled. The distillates were tested for nitrites.

Distillate after 7 hours' growth = 0.0 milligrammes of indole per 100 c.c.							Nitrites
							0
"	"	24	"	"	=0.0	"	+++
"	"	48	"	"	=0.0	"	+++
"	"	4 days'	"	"	=0.7	"	+++
"	"	6	"	"	=0.8	"	0
"	"	9	"	"	=2.0	"	++
"	"	13	"	"	=2.8	"	++
"	"	18	"	"	=7.0	"	0
"	"	27	"	"	=4.8	"	0

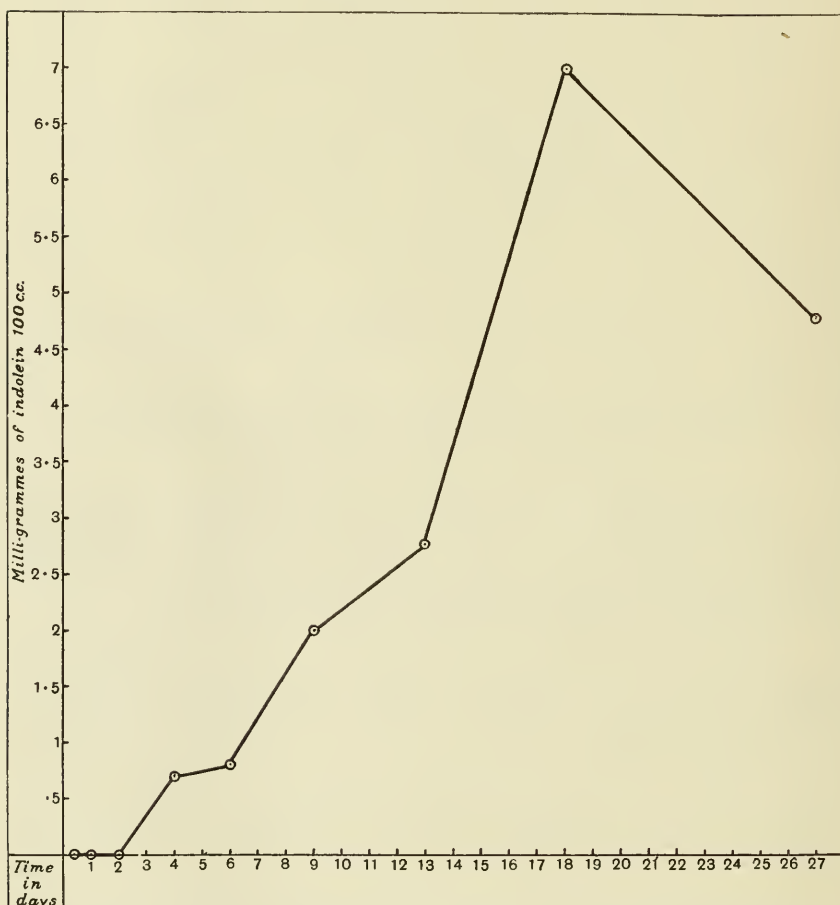


CHART III. Showing the amount of indole present at various stages during the cultivation of the *cholera vibrio* for 27 days at 37° C.



This completed the series and these three curves of indole production are exceedingly interesting, in that they bear a striking resemblance to curves of toxin production. Probably the metabolic processes concerned in the two functions run parallel and certain circumstances which favour toxin formation also favour indole production. They are sufficient, at any rate, to demonstrate the use of this test in the quantitative estimation of indole.

It can also be demonstrated that the presence of lactose and glucose inhibit the production of indole by bacteria. *Bacillus coli*, grown at 37° C. for five days in 100 c.c. peptone beef broth containing 2% lactose, and then distilled, failed to show the least trace of indole. In a similar experiment, with 2% glucose in the broth, only a very small quantity of indole was produced. Here again there is a parallelism between indole production and toxin formation, as the latter, in the case of both the Diphtheria and Tetanus bacillus, is also inhibited by the presence of excess of glucose.

Skatole gives with this reagent a violet colour, which, on standing, becomes deep blue (the appearance of the blue colour is hastened by the addition of the persulphate). Only on one occasion have I found skatole in bacterial cultures, and that was in a broth culture of *Bacillus typhosus*, freshly isolated from the blood.

It may be added in conclusion, that, for laboratory purposes, this test for indole is exceedingly useful. There is no doubt about the result, it shows the presence of very small amounts of indole, and it is easily applied. In the routine examination of organisms isolated from water and from milk, we can say quite definitely in 24–48 hours if indole be present, a great saving of time, as it was customary to wait five days before applying the nitrite and sulphuric acid test.

#### SUMMARY AND CONCLUSIONS.

1. The high estimate formed by Böhme of the utility of this test has been fully confirmed.
2. Other substances, such as skatole-carboxylic acid, which tended to confuse the results of the older method, do not give an indole-like reaction with this reagent.
3. The test is more delicate, and the time required to ascertain the presence of indole is shortened.

4. The method lends itself to accurate colorimetric quantitative estimations, and the amount of indole present at various stages during the cultivation of certain organisms has been determined.

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# ON VARIATION IN WEIGHT OF NORMAL GUINEA-PIGS IN RELATION TO THE ESTIMATION OF FREE DIPHTHERIA TOXIN.

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IN the foregoing paper (p. 512) relationships have been established between loss of weight in test guinea-pigs and dose of diphtheria toxin injected. In connection with the above, the normal increase of weight during the period of observation, the effect of the animal ceasing to eat, or any change of diet, had to be taken into consideration. The following note records observations on normal increase of weight, with constant and variable diet, on healthy animals.

## *Method.*

Ninety-four guinea-pigs were sorted out into batches of five individuals (one batch consisting of 4), so that the companion pigs did not differ from one another by more than 5 grams. Each batch was placed in a separate and similar cage and all were fed regularly at 7 A.M. and 4 P.M. daily. The pigs were weighed regularly between 11 A.M. and noon, and the weight of each individual noted daily, a spring balance being used, the divisions on the scale of which would indicate a difference of 5 grams.

## *Normal Increase in Weight.*

All the pigs were fed with oats at 7 A.M. and with cabbage and oats at 4 P.M. for a week. The number of pigs and the mean values for the normal increase are given in Table I for five days, two days being allowed for the animals to become acclimatised to their surroundings,

diet, the relatively high temperature prevailing, viz. 16° C. at midnight to about 30° C. at midday, and to recover from a railway journey, diet of unknown character, etc. From the figures it is probable that, for guinea-pigs of 160 to 210 grams initial weight, the rate of increase in weight is approximately the same and is nearly a straight line relation, *i.e.* the increment is proportional to the time. The absolute value of the increment per diem, under the above circumstances, was 7 to 9 grams in the two most divergent series, with the exception of one set, in which only four pigs were classed, where the value is approximately 6 grams. I conclude, therefore, that for guinea-pigs used as indicators of free diphtheria toxin the normal increase in weight will be practically independent of the difference in absolute weight of the test animals, provided this difference does not exceed 60 grams, and further that, as the daily increment probably decreases slowly with increasing absolute weight of the test animals in an hyperbolic relation, the value for guinea-pigs of 250 to 260 gms. will be in the neighbourhood of 6 gms. per diem.

TABLE I.

*Average weights of guinea-pigs showing increment during 5 days.*

No. of pigs	0	1	3	4	5
7	210	216	234	243	245
23	202	210	229	235	241
13	191	200	219	224	228
19	183	190	209	215	219
16	173	180	200	204	209
9	163	170	186	194	203
4	150	151	168	180	180

*Effect of Temporary Change of Diet.*

Of the entire series of pigs, 29 were retained on the diet above described and 65 pigs were given a single meal in which grass was substituted for cabbage, and then the original food continued for four days. The pigs would not at first eat the grass so that the effect was striking, for the average per pig of the cabbage group increased by 7 gms., 7 gms., 7 gms. and 5 gms. on succeeding days, whereas the grass group decreased by 10 gms. per pig on the first day and on substituting cabbage, increased on the succeeding days by 12 gms., 6 gms. and 6 gms.; being at the end of this period 6 gms. lower in average weight than the pigs of the cabbage group. Unless a constant diet be employed during the test period of diphtheria toxin any relation between lethal dose and weight will be seriously affected.

*Effect of Continued Change of Diet.*

Many of the pigs originally closely approximating in weight had increased in weight to a much greater extent than others, although subject to the same food conditions, and consequently it was considered advisable to sort out the animals into groups of five, the individuals of each batch differing, as before, by not more than 5 gms. This selection necessitated, for many pigs, a change of cage and of companions, and the effect was that the majority of these pigs lost an average of 5 gms., and in the case of certain animals 10 gms. It will be seen from this that very slight changes in the conditions may interfere with the dose-weight relation. Other slight changes produce equally marked effects. In order to obtain a fairly uniform condition, all the pigs were now kept on cabbage and oats for a week. The cages were then separated into two groups, each containing batches of pigs corresponding closely in weight, the total number of pigs selected for each series being 36. One group was fed on oats and cabbage and the other on oats and grass for seven days. The average weight of each group at the initial weighing being taken as the origin, the total changes in weight for this period are given in Table II.

TABLE II.

*Change in weight during 9 days on diet.*

A = Cabbage and Oats; B = Grass and Oats.

1	2	3	4	5	6	7	8	9
A 0·5	A 5	A 10·5	A 17	A —	A 21	B 27	B 21	22
B 4	B - 4	B - 1	B 5·5	B —	B 12	A 13·5	A 24	35·5

The figures (Table II) show that the effect of substitution of grass for cabbage is attended by an initial marked diminution in the weight of the animals. After two days the pigs fed on grass begin to increase in weight at the same rate as those fed on cabbage, although owing to the initial depression they show no tendency to attain an equal absolute weight. On the seventh and eighth days the grass group was given cabbage and the cabbage group was placed on a grass diet. The change from cabbage to grass produced a fall on the eighth day, followed by a slow rise on the ninth, whereas the change from grass to cabbage gave rise to a very great increment in weight on the eighth and ninth days, the original grass series being at the end of this period 13·5 gms. heavier than the original cabbage series.



*Effect of other Changes in Diet for One Day.*

Ten guinea-pigs which had been fed on grass for a week were given dry grass and oats and showed on the following day an increment in weight of 4 gms. per pig.

Ten pigs of similar weights and also accustomed to a grass diet were supplied with dry grass, oats and a liberal supply of water in separate troughs; in this case the average increment was 9.5 gms.

Under the same conditions, dry grass, oats and minced beef gave a rise in weight of only 4 gms.

The pigs did not eat the beef.

Further experiments with beef alone led to a loss in weight of 21 gms., as however it was found that the pigs had not eaten the beef, the result is obviously due to no food ingested.

This decrease of 21 gms. per day is approximately of the same magnitude as the decrements obtained on the injection of lethal doses of solutions containing free diphtheria toxin, which lends support to the view that the latter largely represent starvation curves.

In conclusion, the normal variation of the guinea-pig as regards weight seems, to me, to give an experimental error of at least 10% in the estimation of lethal doses from decrement in weight under favourable circumstances, and under ordinary conditions the error is probably two or three times this magnitude.

## A CONTRIBUTION TO THE BACTERIOLOGY OF POST-SCARLATINAL DIPHTHERIA.

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THE basis of this paper is the analysis of the bacteriological examination of the fauces of a series of 1019 patients admitted to hospital as cases of scarlatina and a consideration of their subsequent clinical histories. A practice was made of swabbing the fauces of scarlet fever cases without any selection on their admission to hospital. In all cases one bacteriological examination was made and in a number of the cases the examination was repeated.

### *Method of carrying out the Examination and Criteria employed for determining the presence of Bacillus diphtheriae.*

The fauces were swabbed with a sterile cotton-wool swab while the patient was still in the receiving room, so as to obviate any possibility of infection in the hospital. The swab was immediately rubbed well on the surface of a solidified blood serum tube. The tube was incubated at 37° C. and examined after 18 hours. Films were made and stained with Löffler's methylene blue, and in those cases in which there was a doubt as to the character of an organism resembling the diphtheria bacillus separate films were made and stained by Gram's method, by Neisser's stain and by toluidene blue. With this last stain true *B. diphtheriae* stain, light blue, the granules being a deep purple and very definitely outlined. Diphtheroid organisms show polar staining of much less definite character—in most cases staining almost uniformly.

*Results of the Examinations.*

Of the 1019 cases the throat was examined in 1017 and the nose in 165. The examination of the nose was omitted in the majority of the cases. It is recognised that the results would have been more valuable had such an examination been made throughout the whole series.

Of the total 1019, *B. diphtheriae* were found in 75 cases and diphtheroid organisms in 12. That is to say 7·36% of cases of scarlet fever examined on admission showed the presence of *B. diphtheriae* in the nose or throat on one examination and 1·07% showed diphtheroid organisms (*B. Hofmanni*).

These occurred thus.

In the throat 1017 cases examined :

59 or 5·8% showed *B. diphtheriae*.

7 or ·68% showed diphtheroid bacilli.

In the nose 165 cases examined :

21 or 12·72% showed *B. diphtheriae*.

8 or 4·84% showed diphtheroid bacilli.

Comparative results are available. In two of the other hospitals of the Metropolitan Asylums Board the following results were obtained.

In the throat: (a) One series of 203 cases. 5·41% with diphtheria bacilli and 1·2% with diphtheroid<sup>1</sup>.

(b) A series of 87 cases. 6·8% with diphtheria bacilli<sup>2</sup>.

In the nose: (a) A series of 202 cases. 12·37% with diphtheria bacilli and 53·7% with diphtheroid<sup>1</sup>.

(b) Lambert Lack (1898), in a series of 100 cases in the out-patient department of a large general hospital, found diphtheria bacilli in 13% of the noses examined and diphtheroid organisms in 52%<sup>3</sup>.

These results agree closely with those obtained in the present series as far as true diphtheria bacilli are concerned, but the presence of diphtheroid organisms in more than half the noses examined is not in agreement with the results obtained during the present investigation. This discrepancy is probably due to a wider interpretation by these writers of the term "diphtheroid." The term diphtheroid in this paper refers to the class of micro-organisms usually known as Hofmann's bacillus.

<sup>1</sup> Report Metrop. Asylums Board, 1900.

<sup>2</sup> Trans. Epid. Soc. London, vol. xv.

<sup>3</sup> Med. Chir. Trans., vol. lxxxii.

That is to say, then, that of the 1019 cases 75, or 7·36% were harbouring the diphtheria bacilli and 12 or 1·07% other organisms, presumably (in the absence of further bacteriological examination) of the same type.

932 cases were free from such organisms as far as could be seen in one examination.

*Subsequent History of the Cases Examined.*

Of the 932 cases six developed clinical diphtheria.

Five of the faucial type on the 9th, 22nd, 46th, 48th and 50th days of scarlet fever.

One of the laryngeal type on the 38th day—this required tracheotomy but recovered.

These six cases, or 64% of “bacilli free” cases, were presumably cases of hospital-contracted diphtheria.

Of the 75 cases which showed the presence of diphtheria bacilli on admission four cases, or 5·3%, developed clinical diphtheria, three cases occurring on the 4th, 5th and 31st days—the last one of which died of diphtheria. The fourth case is interesting in that it showed diphtheria bacilli in the nose only and not in the throat. It developed typical faucial diphtheria on the 7th day.

There were three other cases which are worth mentioning.

(a) Diphtheria bacilli in the nose only on admission—on the 31st day there were diphtheria bacilli in the nose and throat—on the 44th day pharyngitis occurred, with no formation of membrane.

(b) This case was certified as diphtheria but was diagnosed scarlet fever on admission and showed diphtheria bacilli in the nose only. There was some paralysis of the soft palate on 44th day.

(c) Diphtheria bacilli in the throat only on admission—the patient developed tonsilitis with no membrane on the 26th day.

The throats of these 75 cases presented on admission no evidence on examination of the presence of the bacilli—they differed in no way from the ordinary variety of throat conditions seen in scarlet fever.

Of those cases, then, which showed no bacilli on admission 64% contracted diphtheria, while of those which had already diphtheria bacilli in the throat or nose 5·3% contracted diphtheria. This may be interpreted as indicating that many of those cases of post-scarlatinal

diphtheria apparently contracted in hospital are not really cases of hospital infection, but are due to an attack on the organism by bacilli already present—the scarlet fever having possibly produced a condition of lowered resistance. It is, further, interesting to note that the cases which had diphtheria bacilli on admission developed the clinical manifestations on the 4th, 5th, 7th and 31st days of scarlet fever, while those which were “bacilli free” showed clinical diphtheria at later stages, viz. 9th, 22nd, 46th, 48th and 50th days. Whether this interpretation be accepted or not, the results are sufficiently striking to justify the routine bacteriological examination of every case of scarlet fever admitted and the continuance, in those cases in which bacilli are present, of routine throat treatment well into the stage of convalescence and even right up to the time of discharge from hospital. That one case should have bacilli in the nose only and yet develop faucial diphtheria supports the routine examination of the nose as well as of the throat.

It is striking that of the 75 cases which had diphtheria bacilli on admission only four, or 5·3% (or if we include the three doubtful cases mentioned above seven, or 9·3%) developed clinical diphtheria. That is to say that the possible effect of scarlet fever in raising the virulence of the diphtheria bacilli (or lowering the resistance of the patient) was only seen at the outside in 9·3% and only definitely in 5·3% of the patients in which all the conditions for such a result were already present.

Of the 12 cases which showed Hofmann’s bacilli on admission four showed these organisms in the throat only; five in the nose only; and three in both throat and nose—in none of these cases was there any clinical manifestation that could be associated with their presence, nor were any true diphtheria bacilli found in these cases on repeated examinations.

#### *Persistence of the Bacilli.*

In 29 cases repeated examinations were made to determine how long the bacilli remained in the throat while daily treatment of the throat was being carried out with antiseptic lotions. The periods during which the bacilli remained present were in days: 2, 2, 2, 2, 2, 3, 3, 4, 5, 9, 9, 11, 11, 15, 16, 16, 17, 18, 19, 22, 23, 23, 26, 28, 31, 32, 33, 34, 43. In none of these cases did there develop any clinical evidence of diphtheria. In 11 cases the bacilli disappeared under treatment before the 10th day, and in 19 cases before the 20th. In six other cases, however, the bacilli were still present after 16th, 26th, 38th, 43rd, 45th and 122nd days. In these cases it was not possible, because of the



transfer of the patients to a convalescent hospital, to continue the examination to determine the time of disappearance of the bacilli. In only one of the six were there any clinical signs, that is the one in which they were still present after 43 days—this was the same case referred to above, in which tonsillitis occurred on the 26th day.

The conclusion is, then, that in most of these cases the bacilli quickly disappear under treatment, but that in other cases they may remain long periods, in one case so long as 122 days.

There is still another aspect to be considered. That is that of the 932 cases which showed no diphtheria bacilli on admission there was a total of 28 cases (including the six cases of clinical diphtheria) which subsequently showed the presence of such bacilli.

These 28 cases can be sub-divided thus:

A. Nineteen cases in which the nose was not examined but no diphtheria bacilli were present in the throat on admission.

(i) In 5 cases diphtheria bacilli were found associated with purulent rhinorrhoea on the 8th, 35th, 41st, 44th and 62nd days of scarlet fever.

(ii) In 11 cases diphtheria bacilli were found in the throat, five cases with no clinical signs but bacilli found on the 2nd, 3rd, 13th, 14th and 43rd days.

Six cases, as above with clinical diphtheria on the 9th, 22nd, 38th, 46th, 48th and 50th days of scarlet fever.

B. Nine cases which showed no diphtheria bacilli in nose or throat on admission.

(i) Two of these showed bacilli in both nose and throat on the 30th and 61st days of scarlet fever respectively.

(ii) Five showed bacilli in the nose only on the 34th, 42nd, 45th, 48th and 51st days—all of these had purulent rhinorrhoea but no ulceration or membrane on the septum or the turbinates.

(iii) Two showed bacilli in the throat only on the 29th and 61st days respectively—the latter of these showed the persistence of the bacilli in the ear discharge till the 139th day.

It may be objected that the comparison above of 5·3% of clinical diphtheria cases in which bacilli were present on admission with 64% of apparently hospital-contracted clinical diphtheria is not a just one, but that all bacteriological infection, whether followed by clinical diphtheria or not, should be regarded as of hospital origin.

Even if this is done and the whole 28 cases included it makes 2·75% as against 5·3%, and there is abundance of reliable authority for assuming

that the bacilli were probably overlooked on the first examination, and in 19 of the cases it is possible that the bacilli were present in the nose.

*Conclusions.*

The most interesting facts brought out by this investigation are:

1. That of cases of post-scarlatinal diphtheria with clinical signs the percentage of those which come into hospital with bacilli already present is more than eight times as high as the percentage of those which are presumably infected in hospital.

2. That while in the majority of the cases which are harbouring diphtheria bacilli on admission, the bacilli rapidly disappear under treatment yet, in a certain small percentage, the bacilli may persist for a long time, though not necessarily producing clinical manifestations.

3. That of those cases which had diphtheria bacilli present on admission less than 10% (on the widest calculation) developed clinical signs and only 5.3% developed typical diphtheria.

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## THE RELATIVE FREQUENCY OF VARIOUS TYPES OF STREPTOCOCCI IN SCARLATINA.

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### *The Streptococci obtained in Scarlet Fever.*

It has long been well established that during the height of scarlet fever streptococci flourish and become greatly predominant in the pharyngeal and buccal mucus. To quote only a few of the many recorded observations.

Lemoine (1895) in the mucus from 117 throats in scarlet fever found streptococci alone 93 times and with other organisms in 14.

Baginsky and Sommerfeldt (1900) in 701 cases found that streptococci were absent only five times.

The latest results are those obtained by Ruediger (1906). He inoculated (with broth in which the swab used to collect the material had been washed) blood-agar tubes, from which he made plates. On the medium the streptococci grew as grey colonies surrounded by a clear area of haemolysis. Pneumococci grew as green colonies and could thus be distinguished from streptococci. The colonies obtained were picked off and confirmed by further morphological and cultural investigation. Of 75 throats thus examined during scarlet fever, 2 showed only streptococci, 31 showed 60—95% of streptococcal colonies, 21 showed 15—40% streptococci, and 20 showed streptococci and pneumococci.

Ruediger makes this statement. "As a rule the streptococcus colonies greatly predominate over the other colonies when the inflammation of the throat in scarlet fever is pronounced, and they rapidly decrease in number with the subsidence of the throat

symptoms....When the throat symptoms are mild the proportion of streptococcal colonies to other colonies is quite small."

Ruediger further gives the results of his examination of 51 normal throats. "Streptococci were present in 30 out of 51 cases examined but were never present in large numbers and were entirely absent in 21 cases." The fact of this almost invariable preponderance of streptococci in the throats of early cases of scarlet fever agrees with the writer's experience. In over 250 plates made and examined the streptococci were present in nearly every case in much greater numbers than all the other types of colonies together.

Assuming the fact of an increase in the number of streptococci taking place in the throat during the first few days of scarlet fever as having been demonstrated, the object of this enquiry was an attempt to discover whether these streptococci showed any uniformity in type.

For this purpose the organic substances introduced by Gordon (1905) were employed and the streptococci were grouped according to the results produced by their growth on these media. The formula of the media used was peptone water 75%, beef broth (made from bovine heart muscle) 25%, and in this was dissolved 1% of the test substance.

The streptococci examined included those obtained from abscesses, from the nasal discharge and from the tonsils during scarlet fever. The pus was obtained from the abscesses by inserting a sterile hypodermic needle after sterilizing the skin and removing a few drops of pus, which were grown for 18 hours in broth and then plated off on agar in three dilutions of the broth.

In order to reduce to a minimum the amount of contamination by the ordinary salivary organisms when obtaining specimens from the throat the mouth was first swabbed as dry as possible with cotton-wool. All debris and secretion were then rubbed off the surface of the tonsil with a sterile cotton-wool swab until the mucous membrane just began to bleed. Then a second sterile swab was at once used and soaked in the tonsillar secretion. This swab was then lightly rubbed over the surface of an agar plate. A glass rod bent at right angles was then sterilized and rubbed over this plate, then on to a second plate and straight on to a third. The result on the third plate was a very limited number of colonies (in most cases entirely streptococcal) which could be picked off and examined. From three to five single colonies were taken from each plate and grown separately in broth for 48 hours at 37° C. At the end of 48 hours films were made and examined under the microscope. Any that did not show a uniform morphological type, or, at least, nearly uniform,

were discarded. Then from the broth culture the various chemical media were inoculated. In all 19 strains were examined from abscesses, 80 strains from throats, 1 strain from a case of scarlet fever post-mortem, and 1 from a case of rhinorrhoea within the first day after the onset of the discharge.

These 101 different specimens of streptococci gave the following results with the above tests:

(a) The whole series failed to reduce neutral-red after three days' growth at 37° C. under strictly anaerobic conditions.

(b) Every member of the series grew on gelatin in two days at 22° C.—none liquefied the gelatin, but the amount of growth shown in two days varied within moderate limits.

(c) As a general rule—with only a few exceptions—the streptococci fermented saccharose and lactose.

The remaining reactions need to be considered more in detail.

To take first those streptococci obtained from abscesses. These represent probably the streptococci which invade the general circulation and which, any serum which aims at the improvement of "septic" symptoms in scarlet fever, must attack.

The 19 strains present four distinct types.

No. of specimens	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin at 22° C.
13	—	+	+	—	—	+	—	A	+
4	—	+	—	—	—	+	—	—	+
1	—	+	+	—	—	+	+	A	+
1	—	+	+	—	—	+	—	A.C.	+

A. C. means the production of acidity and clotting in litmus milk.

A means acidity only.

+ = the production of acidity except in the case of gelatin where it means simply "growth."

It will be seen then that the predominant type of streptococcus found in the abscesses examined was one which fermented saccharose, lactose and salicin, which produced acidity but no clotting in milk and which left the other media unaffected. In all the media growth was quite vigorous. These abscesses occurred between the 8th and 34th days of disease. The fact that 19 specimens obtained from abscesses could be divided into four distinct types suggests that if favourable results are to be obtained from serum therapy it will be by the use of a polyvalent serum.



*Rhinorrhoea.*

One strain fermented lactose, saccharose, salicin and mannite and produced only acidity in milk.

*Streptococci from Throats.*

The following table includes all those strains of streptococci obtained by the method described from the throat in scarlet fever.

TABLE A.

Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin	No. of specimens
-	+	+	-	-	+	-	A	+	40
-	+	+	-	-	+	+	A	+	13
-	+	+	F	-	+	-	A	+	2
-	+	+	+	+	+	-	A	+	1
-	-	+	-	-	+	+	A	+	1
Fermented Glucose only									2
-	+	+	-	+	-	-	A	+	1
-	+	+	-	-	-	-	A	+	1

TABLE B.

-	+	+	-	-	+	-	A. C.	+	6
-	+	+	-	-	-	-	A. C.	+	5
-	+	+	+	-	-	-	A. C.	+	4
-	+	+	+	+	+	+	A. C.	+	1
-	+	+	+	+	+	-	A. C.	+	1
-	+	+	-	F	+	-	A. C.	+	2

In Table A are included all those that do not clot milk but produce only acidity. In Table B are included those that both produce acidity and clot the milk. In both tables the letter F is intended to indicate that a faintly acid reaction was produced after five days.

(These 80 streptococci from the throats were isolated from 25 patients between the second and ninth days of disease with one exception, which was obtained on the 26th day of the disease. The colonies were picked off quite indiscriminately from the original plates.)

*Streptococcus obtained Post-mortem.*

This strain was obtained from a case of scarlet fever which was typically of the "Toxic" type. Death occurred on the third day of disease, a rigor having taken place about six hours before death.

Cultures were made from the blood removed from the left ventricle in a sterile pipette, from the splenic pulp and from the liver. Two sets of blood cultures were taken from the heart—both remained sterile. The culture from the liver showed a mixed staphylococcus and *Bacillus coli* culture. The spleen showed a pure streptococcal culture. This streptococcal culture was plated out on agar and single colonies examined. Morphologically it was of "medius" type—it grew in broth with a sandy sediment (type B)<sup>1</sup>—grew fairly well on gelatin at 22° C. in two days, produced acidity but no clotting in milk, and fermented saccharose, lactose, salicin and mannite, but showed no reduction of neutral-red.

Its pathogenicity was tested on mice and it produced a local abscess at the site of injection in four days and death of the mouse on the seventh day. The streptococci could not be recovered from the heart or liver, but only from the abscess of the mouse.

To compare, then, the results obtained by these fermentation tests on the various streptococci. First let us take the two types shown below, which between them include the great majority of the specimens examined. It will be seen that they are closely similar, the differential test being the power of fermenting mannite.

	No. of specimens	Neutral red	Saccha- rose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin
Abscess	13	—	+	+	—	—	+	—	A	+
Throat	40	—	+	+	—	—	+	—	A	+
Abscess	1	—	+	+	—	—	+	+	A	+
Throat	13	—	+	+	—	—	+	+	A	+
Rhinorrhoea	1	—	+	+	—	—	+	+	A	+
Cadaver	1	—	+	+	—	—	+	+	A	+

The fact that not one of these 101 cases reduced neutral-red is striking, several controls were made with streptococci from normal saliva on the same neutral-red media and they produced a reduction.

Gordon (1905) describes five samples of streptococci obtained by him from scarlet fever cases—three of these gave positive reactions to saccharose, lactose and salicin only, and two gave positive reactions to the same three reagents as well as to mannite. Thus all the five specimens belong to the same two groups as the majority of the strains in this series.

Andrewes and Horder (1906) have collected 1200 specimens of streptococci subjected to these differential tests. Thirty-three of these

<sup>1</sup> *Vide infra.*

strains were obtained from scarlet fever cases, and these fall under the classification used by those authors as follows:

<i>S. pyogenes</i>	...	...	...	...	12
<i>S. salivarius</i>	...	...	...	...	1
<i>S. anginosus</i>	...	...	...	...	20

The *S. pyogenes* group corresponds with that into which the large majority of the cases in this series fall. Of the 20 strains included in the *S. anginosus* group, nine do not reduce neutral-red and the reactions given by these nine correspond closely with the reactions given by members of the present series.

A careful repetition of the nine tests, in many of the cases after long intervals—in some cases three months—corroborated the assertion of these other writers that each strain of streptococcus preserves its characteristics unchanged.

#### *Culture appearances in Broth.*

In each case a note was kept of the way the streptococcus grew in broth.

The types of growth are divided into four.

Type A. Uniform turbidity with little deposit.

B. Clear broth with sandy deposit.

C. Clear broth with small flocculent masses adhering to the bottom and sides of the tube.

D. Clear broth with larger floccular masses on the bottom of the tube.

The following tables show the chemical reactions given by these different types.

It will be seen from these tables that there is no close connection between the type of growth in broth (the only method of separating streptococci before the introduction of these "nine tests") and the reactions given with the tests under discussion. It is interesting to

#### *From Abscesses.*

	Neutral red	Saccha- rose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin	No. of specimens
Type B	—	+	+	—	—	+	—	A. C.	+	1
	—	+	+	—	—	+	—	A	+	8
	—	+	—	—	—	+	—	—	+	4
Type C	—	+	+	—	—	+	—	A	+	5
	—	+	+	—	—	+	+	A	+	1

*From other sources.**Type A.*

Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Gelatin	Milk	No. of specimens
—	+	+	—	—	+	—	+	A. C.	2
—	+	+	—	—	—	—	+	A. C.	4
—	+	+	+	—	—	—	+	A. C.	3
—	+	+	+	+	+	+	+	A. C.	1
—	+	+	—	—	+	—	+	A	2
—	+	+	F	—	+	—	+	A	1

*Type B.*

—	+	+	—	—	—	—	+	A. C.	1
Glucose only							+		2
—	+	+	—	—	+	—	+	A	4
—	+	+	—	—	+	+	+	A	1

*Type C.*

—	+	+	—	—	+	—	+	A. C.	4
—	+	+	—	F	+	—	+	A. C.	2
—	+	+	—	—	+	—	+	A	30
—	+	+	—	—	+	+	+	A	14
—	+	+	F	—	+	—	+	A	2

Five other varieties with one member each.

*Type D.*

—	+	+	+	+	+	+	+	A. C.	1
—	+	+	—	—	+	—	+	A	4

note, however, that in the group, Type A, which so far as morphology is concerned coincides with *Streptococcus brevis*, 10 of the 13 members clotted milk, while in the group, Type C, morphologically *Streptococcus longus*, 51 of the 57 did not clot milk.

That is to say the majority of the streptococci found in scarlet fever throats correspond with the *S. longus* type.

This series of streptococci then shows:

(1) That 50% of the throat streptococci examined give identical results on these selected media.

(2) That 68.4% of the streptococci obtained from the abscesses give identical results.

(3) That the majority of throat streptococci in scarlet fever are of the same type as the majority of streptococci obtained from abscesses in various parts of the body.

(4) That if we include the specimens that "fermented" mannite, then we find that 69% of the streptococci obtained from throats,

abscesses, nose and cadaver in scarlet fever are of identical type and correspond with the specimens isolated by Dr Gordon from throats, abscesses, cadavers, ears and cervical glands in scarlet fever.

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## ON THE SUPERSENSITATION OF PERSONS BY HORSE-SERUM.

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Dr J. R. Currie's interesting and instructive article in the January, 1907, number of the *Journal of Hygiene* induces me to publish a short account of certain cases that have come under my observation at the Eastern Fever Hospital. They afford additional information to that given in his paper.

Most of those who have had more than a scanty experience of the antitoxin treatment of diphtheria, are familiar with the phenomena of supersensitisation as they manifest themselves in the human subject. It is some years now since I became acquainted with them. At first I was inclined to attribute the symptoms to some peculiarity of the serum employed; but subsequent observations showed that this view was incorrect, and that the essential factor in their production appeared to be a fresh injection of serum after an interval of some duration (weeks or months).<sup>1</sup> I sent an account of three well-marked cases of what is now termed the "immediate reaction" to the Committee of the Clinical Society which was investigating the claims of the antitoxin treatment of diphtheria at the time; but the report of that Committee was in the press, and so the notes of the cases were published in the appendix to the report without comment<sup>2</sup>. I have since met with a few similar cases, which are included, with those reported to the Committee of the Clinical Society, in Table I.

As von Pirquet and Schick and Dr Currie have shown, and as has been observed experimentally in animals, it is not the number of injections of serum that is of importance, but the length of time that

<sup>1</sup> See *Encycl. Medica*, Vol. III. p. 18.

<sup>2</sup> See *Clin. Soc. Trans.* Vol. xxxi.

elapses between the first injection or series of injections (extending over a few days), and the second injection or series of injections. My cases afford some evidence as to the length of this interval. At the Eastern Hospital patients have repeatedly been injected with serum on two, three, four or more occasions on successive days<sup>1</sup>; and I have never had any reason for supposing that the serum phenomena were earlier in their onset in consequence. This view is supported by Dr Currie's observations; though he does suggest that the later injections of a series may determine the appearance of a rash which would otherwise have remained undeveloped. To convert this suggestion into a certainty, or to disprove it, requires the analysis of a larger number of cases than he gives. The appearance of a rash on the fifth day after a single primary injection is by no means uncommon; and it is still less uncommon on the sixth or seventh days. Hence if injections are given in any particular cases daily for a week, and a rash appears on the sixth or seventh day, it cannot be certain that its appearance has been determined by any of the later injections.

There is also another fact which makes the question more difficult of solution, and it is one to which Dr Currie does not refer; namely, that one single injection may produce two (and on rare occasions even three) distinct rashes (*e.g.* urticaria and erythema circinatum) separated by a definite interval in which there is no rash nor other serum phenomena. Cases 31, 47, 54 and 74 in Table I are examples of this. (See also the Clinical Society's Report.) The explanation of this fact that has offered itself to me is the mixing of the sera of two or more horses in order to obtain a serum of an antitoxic value that is between the two extremes. It is customary to attribute the occurrence of a reaction largely to the idiosyncrasy of the patient injected; but the idiosyncrasy of the horse which supplies the serum has certainly to be considered, with regard both to the frequency and character of the rashes. The fact that different sera will produce different serum-phenomena was noticed by the late Dr J. W. Washbourn, Mr A. H. Card and myself in the first series of cases of diphtheria that were treated with serum in this country<sup>2</sup>. I think it is not unreasonable to assume that if the serum of one horse gives rise mostly to urticaria (which is usually somewhat early in its appearance compared with *E. circinatum*), and of another mostly to *E. circinatum*,

<sup>1</sup> I have notes of a few cases in which patients received up to eight injections of serum within a few days without any hastened serum-reaction, sometimes without any reaction at all. One patient received 200 c.c. in eight injections during 11 days, and had no reaction.

<sup>2</sup> *Clin. Soc. Trans.* Vol. xxxiii.

then a mixture of the two may as the result of a single injection produce the one rash followed at a distinct interval by the other. (Case 3 of Dr Currie's series may be thus explained.) So that the practice of mixing sera vitiates the conclusions that might be drawn as to the manifestation and time of occurrence of rashes from an analysis of cases in which there had been several injections on successive days.

One thing, however, appears to be certain, that it is extremely unusual for serum-phenomena to manifest themselves within three days of a single injection or the first of a series of injections (provided that the patient has never had serum some weeks, months, and occasionally even years before). My experience on that point quite confirms that of Dr Currie as shown in diagram B in his paper. But if the patient has had one or more injections of serum some considerable time previously, then he may get a serum-reaction earlier than usual.

Now the cases that present an interval, usually of some length, between two injections or series of injections, are those in which the patients are treated with serum for a relapse or second attack of diphtheria (or suspected diphtheria), after having been treated with serum in the first attack. I happen to have at hand the notes of all such cases that occurred at the Eastern Hospital during the years 1897, 1898 and 1899, and 1904, 1905 and 1906. The following table, Table I, gives the essential particulars of these cases. As there are some differences between them, the two series are distinguished in the table. Those against which the words "second series" are placed in Column VIII belong to the years 1904, 1905 and 1906, the rest belong to the years 1897, 1898 and 1899.

The cases, 90 in number, are arranged according to the length of time that elapsed between the primary attack and the relapse or second attack, beginning with the case that had the shortest interval.

In Column I are put the numbers applied to the cases for the convenience of reference.

In Column II is stated the number of injections of serum given for the primary attack ; and the days on which the injection or injections were given. The days are reckoned from the first injection, *i.e.* the first day is the day of the first injection. Thus in case 4, 1, 2, 4, mean that one injection was given on the first day (usually the day of admission to hospital), one the day after, and one three days after. One injection was given each day, except in cases 89 and 90.

In Column III are given the times of occurrence and nature of any serum-reaction caused by the injection for the primary attack. Thus

TABLE I.

I	II	III	IV	V	VI	VII	VIII	IX
1	1	None; but see Col. VI	8	—	11, rash; 12, joint-pains (?)	—	—	F. 16
2	1, 3	8, rash	16, 17, 18	—	19, rash	—	—	M. 3
3	1	—	16	—	—	—	Second series	M. 2
4	1, 2, 4	—	18	—	—	—	—	F. 7
5	1	—	18, 22	—	—	—	—	M. 6
6	1	—	19, 20	—	—	—	—	M. 3
7	1, 2	7, rash	19	—	—	—	—	F. 6
8	1	—	23	—	—	—	—	M. 12
9	1	11, rash	23, 24	—	—	—	—	F. 11
10	1	—	24	—	—	—	—	F. 5
11	1	8, rash	25	—	26, rash	37, rash	—	F. 2
12	1	7, rash	26, 27	—	27, joint-pains; 30, rash	—	Second series	F. 37
13	1	—	27	—	—	—	Second series	M. 2
14	1, 2	—	28	—	—	—	—	M. 16
15	1	9, rash	30	—	32, rash	—	—	M. 8
16	1	—	30	—	—	—	Second series	M. 1
17	1	—	31, 36	—	—	—	—	M. 1
18	1	10, rash	32	—	33, rash	—	Second series	F. 4
19	1	9, rash	34	—	—	—	—	F. 2
20	1	—	34	—	—	—	Second series	M. 1
21	1	—	34	—	—	—	Second series	F. 10
22	1, 2	—	35	—	—	—	Second series	M. 7
23	1	10, rash; 12, joint-pains	36	Rash within an hour	41, rash	—	Second series	M. 2
24	1	—	36	—	—	—	Second series	F. 3
25	1, 2, 3	11, rash	38	20 mins., rigor, convulsions; T., 105° F.	39, rash; 42, rash	—	—	F. 5
26	1	8, rash	38	30 mins., rigor; T., 105° F.	39, rash	—	—	F. 6
27	1	—	40	1 hour, rash	—	46, rash	Second series	F. 5
28	1	—	41	—	—	—	Second series	F. 2
29	1	—	42, 43, 44	—	—	—	—	M. 10
30	1	13, rash	42	6 hours, rash; T., 104° F.	—	48, rash	—	M. 3
31	1	5, rash; 8, rash	42	slight shivering at once; 6 hours, rash	44, joint-pains	49, rash & joint-pains	Second series	F. 23
32	1	16, rash	45	—	46, rash; 50, rash	—	Second series	F. 2
33	1	10, rash	46, 47, 48	—	51, convulsions (?)	—	There lapse of diphtheria, began with a severe rigor	M. 2

I	II	III	IV	V	VI	VII	VIII	IX
34 1	—	—	46	—	48, rash	—	Second series	M. 2
35 1	10, rash	—	48	3½ hours, rash	—	54, rash	Second series	M. 4
36 1, 2	8, rash	—	48	—	—	—	Second series	M. 4
37 1, 2	19, rash	—	49	—	50, rash	56, rash	Second series	M. 2
38 1	—	—	49	—	—	56, rash; 59, local abscess	Second series	F. 2
39 1	—	—	50, 51	—	51, rash and collapse before 3rd injection	—	—	F. 3
40 1, 2, 3, 4	—	—	51	15 mins., rash & vomiting; 2½ hrs. rigor	—	—	—	M. 6
41 1, 2, 3, 4	16, rash	—	51	1 hour, rash, vomiting, rigor	—	57, rash	—	F. 3
42 1	13, rash	—	52	—	—	58, rash	—	M. 1½
43 1, 2, 3	9, rash and joint-pains	—	52	—	—	58, rash	—	F. 43
44 1, 2	—	—	53	—	—	—	—	F. 9
45 1	—	—	53	—	—	—	—	M. 11
46 1	—	—	54	—	—	60, rash	—	F. 4
47 1	4, rash; 15, rash	—	54	30 mins., rash & collapse	—	60, rash	—	F. 2
48 1	12, rash	—	55	2 hours, rash	—	—	—	F. 6
49 1	—	—	55	—	—	62, rash	Second series	F. 6
50 1	—	—	57	—	58, rash	—	—	F. 2
51 1	—	—	57	—	—	64, rash	Second series	F. 7
52 1, 2	10, rash; 19, rash	—	58, 66	1 hour after 3rd injection (58th day), rash	68, rash	—	—	F. 1
53 1	—	—	59	—	—	—	—	F. 9
54 1	12, rash; 15, rash	—	59	15 mins., rash, T. 103° F., vomiting	61, rash	65, rash	On the 42nd day the mastoid antrum was opened under chloroform. On the 55th day there were convulsions, followed by a macular erythema on trunk and limbs; cause unknown	M. 2
55 1	—	—	59	—	—	—	Second series	M. 3
56 1	10, rash; 13, joint-pains	—	60	—	—	68, rash	Second series	M. 2
57 1	10, rash	—	63	—	67, rash	69, joint-pains	Second series	M. 3
58 1, 2	9, rash	—	64 (2 lots at interval of a few hrs.)	—	65, rash; 69, rash	—	—	M. 4
59 1, 2, 3	—	—	64, 66	—	—	—	—	M. 5



TABLE I (continued).

I	II	III	IV	V	VI	VII	VIII	IX
60	1, 2	—	65	—	—	—	Second series	F. 3
61	1	15, rash	67	—	—	—	—	F. 6
62	1, 2	—	68	—	69, rash	74, rash	—	F. 5
63	1	4, rash	70	—	—	—	—	F. 4
64	1, 2	10, rash	72	—	74, rash	—	—	M. 6
65	1, 3	—	72	1½ hrs., rigor, col- lapse, T. 103.6° F.	75, rash	78, rash	—	M. 4
66	1	—	72	—	—	—	—	F. 9
67	1, 2	7, rash	75, 76	—	78, rash	—	—	F. 5
68	1, 2, 3	—	75, 76	At once after each of the 4th & 5th injections, a rash	—	—	—	M. 10
69	1, 2	—	77	—	—	—	—	F. 3
70	1, 2	11, rash	78	—	—	—	Second series	F. 3
71	1, 2, 3, 4	16, rash	80	3½ hours, rash	84, rash	—	—	F. 3
72	1	—	85	—	—	—	Second series	F. 2
73	1	—	93	—	—	—	Second series	F. 11
74	1	9, rash; 16, rash	94	—	—	—	—	F. 6
75	1	—	97	—	—	—	—	F. 5
76	1	—	102	—	—	—	—	F. 5
77	1, 2	—	111	—	—	—	—	M. 8
78	1	—	113	—	—	—	Second series	M. 2
79	1, 2	—	123	—	—	—	—	M. 2
80	1	—	139	—	—	145, rash	Second series	F. 4
81	1	13, rash	145	—	—	152, rash	—	M. 4
82	1, 3	9, rash	154	—	155, rash	—	—	F. 4
83	1	—	207	—	—	215, rash	—	F. 2
84	1	—	308	—	309, rash	—	Second series	M. 1
85	1	7, rash	364	2 hours, rash	—	370, rash and joint- pains	—	M. 5
86	1	8, rash	387	—	388, rash	393, rash; 396, rash	Second series	F. 3
87	1	6, rash	416	—	417, rash	—	Injected again for a 3rd attack of diphtheria on 4th day; rash all contested; 4th injec- tion next day	F. 5
88	1, 2	—	529	—	533, rash	—	Second series	M. 1
89	1 (2 lots at inter- val of a few hrs.)	7, rash and local abscess	1165	—	1169, rash	—	Second series	M. 5
90	1 (2 lots at inter- val of a few hrs.)	—	1511	—	1512, rash	—	Second series	F. 5

*Note.*—Cases 16, 20, and 83 were doubtfully diphtheria at both the first and second attack.

Cases 18, 47, 52 and 85 had diphtheria at the first attack, but not at the second attack.

Cases 88 and 90 did not have diphtheria at the first attack, but did have it at the second.

in Case 7, Column III, "7, rash" means that a rash came out on the seventh day (six days after the first injection).

In Column IV is stated the day on which serum was given for a relapse or second attack, reckoning the first day to be that on which the first injection was given for the primary attack. One injection was given on each day except in case 58, where two injections were given at an interval of several hours on the 64th day.

In Column V are shown the time of occurrence and nature of any "immediate reaction," that is a reaction taking place within twelve hours of the first injection for the relapse<sup>1</sup>.

In Column VI are given the dates of occurrence and nature of "accelerated reactions," that is reactions appearing from twelve hours to five days after the first injection for the relapse.

In Column VII are given what may be termed the normal reactions occurring after the injection for the relapse, that is rashes or other symptoms appearing on the sixth or later day from the first injection.

Column VIII is reserved for notes on some of the cases.

In Column IX are given the sex and age of the patient. The age is the age at the time of the primary attack.

It will be observed that in only four of the cases did the reaction due to serum in the primary attack occur within five days of the first injection, within the time, that is to say, of an "accelerated reaction," viz.:—cases 47 (third day after the injection), 63 (ditto), 31 (fourth day), and 87 (fifth day). In all these four cases there was only a single injection. The second attack in case 47 was attended with an "immediate reaction," in case 63 with no reaction, in case 31 with both an "immediate" and an "accelerated reaction," and in case 87 with an "accelerated reaction."

*The Immediate Reaction.* In 17 instances, 18·8 per cent., there was an "immediate reaction." Taking the two series of cases separately it will be found that for the first series of cases, 55 in number during the years 1897 to 1899, the percentage was 23·6; and for the second series, 35 during 1904 to 1906, 11·7; there being 13 cases of this reaction in the first and four only in the second series.

In no instance did an "immediate reaction" commence later than six hours after the injection of serum. There are 22 cases in which the serum treatment for a relapse was resorted to before the 35th day after the first injection in the primary attack, and in not one of them did an

<sup>1</sup> I have not seen an abnormal reaction take place between 6 and 19 hours after an injection; hence I have taken 12 hours as the limit of an "immediate reaction."

"immediate reaction" occur. The earliest instance of this reaction was observed in a patient who was again injected 35 days after the primary injection; the latest in a patient injected 363 days after. But it will be noticed that 16 of the 17 cases of "immediate reaction" occurred in patients who were reinjected 35 to 79 days after the primary injection.

The number of cases is small; but so far as they go the figures show that a person who is submitted to a second serum-treatment within five weeks of the commencement of the first, is not likely to suffer an "immediate reaction." The "immediate reaction" may present severe symptoms. Dr Currie does not appear to have witnessed a case of this sort himself, though he alludes to cases that have been recorded by others; case 25 had a rigor followed by convulsions; case 65 a rigor followed by collapse; cases 26, 31, 41 and 42 a rigor; and case 47 collapse; that is seven of the 17 cases certainly presented severe symptoms. In case 25 the symptoms were very severe. Cases 25, 26 and 41 are the three cases recorded in the Appendix to the Clinical Society's Report. Even when the "immediate reaction" consists of a rash only, the rash is prone to be severe and accompanied by a high temperature. All these cases recovered. In case 68 the patient twice had an "immediate reaction."

As has been stated instances of "immediate reaction" have been less frequent of recent years. The dosage (by volume) of serum was rather larger in the second than in the first series of cases. But I should not be justified in assigning this as a cause of the diminished frequency of the reaction.

Though I have a very extensive experience of serum treatment, I have never seen an "immediate reaction" of any kind after a first injection for a primary attack of diphtheria; and I have never seen convulsions, a rigor, or vomiting due to serum except as part of such a reaction, with the possible, but very doubtful, exception of case 33. Only once do I remember to have seen collapse in connection with serum-phenomena apart from an "immediate reaction," and that was in a case of "accelerated reaction," case 39. Rigors are very uncommon in diphtheria.

The *Accelerated Reaction*. I have placed a ? against two of the instances recorded in Column VI, cases 1 and 33. The rash and joint-pains observed in case 1, ten and eleven days after the first injection may very well have been and most probably were due to it. In two other instances I have seen joint-pains as part of an "accelerated reaction," cases 12 and 31; but I have never seen them as part of an "immediate

reaction." In case 33 the relapse was a severe one and began with a violent rigor lasting for twenty minutes; four hours later an indefinite and transient erythema was noticed on the trunk. Antitoxin was not given till four days after the relapse begun, and it was given on three successive days. The convulsions came on three days after the last injection, and proved fatal. There was no rash. Convulsions, usually fatal, are occasionally seen in severe diphtheria, whether treated or not with antitoxin; in most instances they appear after a few days' illness. I do not think, therefore, that in case 33 they were in any way due to the serum.

Excluding these two cases there were 30 cases of "accelerated reaction," 33·3 per cent. The proportion was higher in the second than in the first series, 37·1 per cent. as against 30·8 (13 in 35 and 17 in 55).

The shortest period between an injection for a relapse and an "accelerated reaction," is one of 19 hours, case 86. In every case except one, case 23, the reaction occurred before the fifth day; in 17 cases (including case 86) it appeared the day after; in 5, two days after; in 2, three days after; in 5, four days after; and in 1, five days after. The earliest instance of this reaction after the first injection in the primary attack was 18 days, case 2 (possibly the reaction was due to this injection); the latest occurred 1511 days after, case 90. This reaction usually presents itself as a rash, with or without pyrexia. In one case there was collapse; and in two joint-pains.

In eight instances the "accelerated" had been preceded by an "immediate reaction."

Rarely is the "accelerated reaction" severe; but in all the instances the rash was more than local; except in the third attack in case 87 (not reckoned in the statistics).

The *Ordinary Reaction after injection for a relapse.* (Column VII.) In 24 instances there was a serum-reaction on the sixth day or later after the first injection for a relapse. It is a curious fact that in 15 of the 24 the reaction occurred on the sixth day, that is rather earlier than the ordinary reaction is usually seen after a first injection for a primary attack. Six of the remaining nine cases occurred on the seventh day, two on the eighth and one on the twelfth. In 15 of the cases there had been an "immediate" or an "accelerated reaction."

Thus though there is a distinct break between the extraordinary ("immediate" and "accelerated") and the ordinary reactions, most of the former occurring on the same day as, or on the day after, the reinjection for the relapse and most of the latter on the sixth and seventh



day after, yet even the ordinary reactions appear in these cases to be hastened when compared with the ordinary reactions after the injection of serum in a primary attack. Indeed it might be stated that there are two varieties of "accelerated reaction," one occurring about five days later than the other.

*Connection between the occurrence of an "immediate" and an "accelerated reaction" and any serum-incidents of the primary attack.* Of the 90 cases 41, 45·5 per cent., had a serum-reaction after the injection given for the primary attack. The percentage of cases with reaction following one injection is 43·3, and following more than one, 50·0. The following table (Table II) shows the relation between the occurrence of an "immediate" or an "accelerated reaction" or both and of a reaction after the serum given in the primary attack.

TABLE II.

	Immediate reaction	Accelerated reaction	Imm.+acc. reaction	No reaction	Total
No reaction after primary attack	2	7	1	39	49
Reaction     "     "     "	7	15	7	12	41
Total	9	22	8	51	90

There was an "immediate" or an "accelerated reaction," or both, in 20·4 per cent. of the cases in which there had been no reaction after the serum given for the primary attack; whereas there was an abnormal (*i.e.* "immediate" or "accelerated") reaction in 70·7 per cent. of the cases in which there had been a reaction at the primary attack.

The following table (Table III) shows the relation between the occurrence of an "immediate" or an "accelerated reaction" and the amount of serum given in the primary attack. During the years under consideration, in both series of cases, serum was given by the 1000 units in varying volume; but it may safely be stated that the larger the number of injections the larger the volume of serum given. During the years 1904, 1905 and 1906 the dosage (by volume as well as by unit) was larger than during the years 1897, 1898 and 1899; but the frequency of injections was less.

TABLE III.

	Immediate reaction	Accelerated reaction	Imm.+acc. reaction	No reaction	Total
One injection	6	11	4	37	58
More than one injection	3	11	4	14	32
Total	9	22	8	51	90

There was an abnormal reaction in 36·2 per cent. of the cases in



which one injection had been given at the primary attack ; whereas the percentage was 56·2 in cases in which more than one injection had been given.

The following table (Table IV) shows the relation between the occurrence of an abnormal reaction in a relapse, and the number of injections and occurrence of a reaction at the primary attack.

TABLE IV.

	Abnormal reaction	No abnormal reaction	Total
One injection followed by } reaction	18 ; 66·6 per cent.	9 ; 33·4 per cent.	27
One injection not followed } by reaction	6 ; 17·3   ,,	28 ; 82·7   ,,	34
Two or more injections } followed by reaction	11 ; 73·3   ,,	4 ; 26·7   ,,	15
Two or more injections } not followed by reaction	4 ; 28·5   ,,	10 ; 71·5   ,,	14
Total	39	51	90

The figures in these tables, though small, go to show that, given an interval of three to five weeks between primary attack and relapse, if a patient has had more than one injection of serum and had a reaction in the primary attack, he is more likely to get an abnormal reaction of some sort if treated with serum in a relapse than is the patient who has had less serum and no reaction in the primary attack.

### *Summary.*

1. There were 90 patients at the Eastern Hospital, who, during the years 1897, 1898, 1899, 1904, 1905 and 1906, were injected with horse-serum for a second attack or relapse of diphtheria, who had been treated with horse-serum at the first attack.

2. Of these 9 had an "immediate reaction," 22 an "accelerated reaction," and 8 had both, 39 in all, or 43·4 per cent.

3. The "immediate reaction" showed itself a few minutes to six hours after the first injection for the second attack.

4. The shortest interval between the first injection at the primary attack and the occurrence of an "immediate reaction" after a reinjection for a second attack was 35 days ; the longest was 363 days.

5. The "accelerated reaction" showed itself 19 hours to five days after the first injection for the second attack.

6. The shortest interval between the first injection at the primary attack and the occurrence of an "accelerated reaction" after a reinjec-

tion for a second attack was 25 days; the longest was 1511 days. In each of these cases the reaction occurred the day after the reinjection.

7. In seven of the cases of "immediate reaction" the symptoms were severe. In all save one of those of "accelerated reaction" they were mild or moderate.

8. In 24 of the cases there was a normal serum-reaction after the reinjection for the second attack; but even this occurred in most cases slightly earlier than is usual after injection at a primary attack.

9. So far as the figures go they show :

(a) that when a patient has had a serum-reaction at the primary attack, he is more likely to have an abnormal reaction after injection at the second attack ;

(b) that the greater the volume of serum given in the primary attack, the more likely is an abnormal reaction to occur after serum at the second attack ;

(c) and that consequently an abnormal reaction is most likely to occur after serum at a second attack when the patient has had a large volume of serum followed by a reaction at the first attack.

There are, however, indications that the volume of serum given at the primary attack may have different effects as regards the determination of an "immediate" or an "accelerated reaction," according to its amount.

## EXPERIMENTAL STUDIES RELATING TO "SHIP-BERI-BERI" AND SCURVY<sup>1</sup>.

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### I. INTRODUCTION.

*On polyneuritis of poultry (polyneuritis gallinarum Eijkman).*

AMONG the diseases which have of late attracted the attention of the official medical authorities of Norway, so-called *ship-beri-beri* takes a prominent place. The symptoms of this disease consist, in the great majority of cases, in weakness and a prominent dropsy of the lower limbs, extending often to other parts of the body. There also exists shortness of breath and other symptoms of a weak heart, causing often sudden deaths from acute paralysis of the heart. But as Nocht<sup>2</sup> in Hamburg and the *Norwegian Ship-beri-beri Committee*<sup>3</sup> have shown, symptoms of *neuritis* of the limbs are comparatively rare. For instance, Nocht was only able to ascertain neuritis in cases from four of his thirty-four beri-beri-ships; and though the investigations of the Committee extended to fifty-seven affected ships, the neuritis was only found in men from four of them. Considering that neuritis is the essential symptom of the beri-beri of tropical countries and Japan, and that dropsy, though present, as a rule is not prominent in cases of this disease, it seems, therefore, rather doubtful whether ship-beri-beri is identical with tropical beri-beri. This doubt, first emphasized by Bullmore<sup>4</sup> and Nocht, is strengthened by the fact that the great majority of patients suffering from the ordinary, *i.e.* dropsical ship-

<sup>1</sup> Read before the Epidemiological Society of London.

<sup>2</sup> *Hansa*, No. 29, 1900, and *Festschrift zum sechzigsten Geburtstage von Robert Koch*, Jena 1903.

<sup>3</sup> *Indstilling fra Beri-beri-Komiteen (Report of the Beri-Beri-Committee)*. Christiania, 1902.

<sup>4</sup> *Lancet*, 22. ix. 1900.

disease, recover as soon as they are able to change their food, which is not the case with the disease of the tropics and Japan.

The subject of this and the following articles will be limited to experiments relating to the dependence of the ship-disease on food, the etiology of tropical beri-beri being outside the field of these investigations.

The recovery of patients suffering from ship-beri-beri is due to *fresh food*. This fact has chiefly been emphasized by Nocht, while the report of the Norwegian Committee contains many corresponding observations. No doubt, some cases do not appear to agree with this observation, which fact, perhaps, favours the idea that “ship-beri-beri” occasionally comprehends more than one disease. This idea may possibly also be supported by the occurrence of cases of neuritis. But in the great majority of cases the effect of fresh animal food, such as eggs or meat, but above all of fresh green vegetables or potatoes, is very marked. For instance, Nocht (*l.c.*) has pointed out that the symptoms regularly disappear within 8—14 days after the patients get fresh food: even after 4—5 days many patients feel able to resume their work.

That ship-beri-beri is very closely connected with food, is further evident from the fact, that the disease appears almost solely on board sailing-ships on long voyages. On account of this fact, first observed by Dr Stian Erichsen<sup>1</sup> in Norway, Nocht has introduced the name “beri-beri of sailing-vessels.” Cases very seldom appear on board steamers on the same waters. It seems natural to explain this difference by the fact that on long voyages sailing-ships take considerable time before they reach a port where they are able to get fresh provisions, whereas this is not the case with steamers.

Apart from the sore gums and haemorrhages in the skin and muscles, which Nocht observed in cases from twelve of his thirty-four ships, but which the Norwegian Committee could not find, it therefore seems probable, that *ship-beri-beri is, in accordance with the opinion of Nocht, a food disease, showing a marked congruence with scurvy.*

That the malady is a form of scurvy (Nocht) or related to it (Nocht and the Norwegian Committee), is also supported by the fact, quoted by Nocht, that cases of dropsy without haemorrhages or sore gums often occur during epidemics of manifest scurvy. For instance, Nocht quotes authors relating a considerable number of cases of this kind in

<sup>1</sup> *Tidsskrift for den norske lægeforening (Journal of the Norwegian Medical Association)*, 1899 and 1901.

the epidemic of scurvy, during the siege of Paris in 1870-71. He has further stated, that such cases often occur during epidemics of scurvy in Russia. I may add that many dropsical cases without sore gums and hemorrhages were also observed during the Crimean war where scurvy was very prevalent<sup>1</sup>. (Some of these Crimean cases were connected with anaesthesia of the feet and looked like "acrodynie" or beri-beri; but as they were sometimes associated with a local gangrene of the feet or with distinct symptoms of scurvy, the opinion prevailed that they were due to the cold of the winter in addition to latent or manifest scurvy<sup>2</sup>.) It may further be mentioned, that many cases of dropsy without sore gums, &c. occur every year besides manifest scurvy on board the French vessels fishing for 5-6 months on the banks of Newfoundland<sup>3</sup>. Finally I may add, that during the first part of the 19th century not only scurvy, but also dropsy without sore gums and haemorrhages was very common in European and American prisons. For instance, besides typhoid fever and consumption, this "prison-dropsy" (*Wassersucht der Gefängnisse*) is stated, in 1847, to have been the most prevalent cause of death in 41 prisons in England, France and North America<sup>4</sup>. In 1857 it caused one half of the deaths in a prison in Breslau<sup>5</sup>; and if it were not that nothing is said about the occurrence of neuritis, some reports from those days recall the descriptions of the Asiatic beri-beri-prisons of our own time<sup>6</sup>.

Proceeding to the question as to *which* faults in diet may be the essential causes of ship-beri-beri, Nocht limits himself to expressing the opinion, that it must be left to the future to decide whether the disease is due to some special sort of starvation or to an intoxication by fermenting food or another poison, or to an autointoxication. The question is more positively answered by Bullmore (*l.c.*), who, basing his opinion on the fact that the disease mostly appears on board ships in the tropics, has started the idea, that the malady is due to an intoxication through all sorts of food, including tinned foods, becoming tainted in

<sup>1</sup> Grellois, *Recueil de mémoires de médecine, de chirurgie et de pharmacie militaire*, vol. xvii. p. 269 a. f.

<sup>2</sup> Macleod, *Notes on the surgery of the war in the Crimea*, Philadelphia and London 1862, p. 8 a. f., cf. also Laveran, *Traité des maladies et épidémies des armées*, Paris 1875, p. 506.

<sup>3</sup> Bonain, *Arch. de médecine navale*, 1907.

<sup>4</sup> Wald, *Vierteljahrschr. für gerichtl. Med.* 1857, vol. xi.

<sup>5</sup> Baer, *Weyls Handbuch der Hygiene*, "*Hygiene des Gefängniswesens*," pp. 42, 43.

<sup>6</sup> Kersandt, quoted by Baer, *Vierteljahrschr. f. öffentl. Gesundheitspflege*, 1876.



consequence of the hot climate. This idea has also been adopted by the Norwegian Committee, which also founds its opinion on the corresponding theory as to the etiology of scurvy. According to the Committee the principal cause of the disease is tainted tinned meat and fish, since Stian Erichsen (*l.c.*) has shown, that the disease did not become prevalent on board Norwegian ships, before tinned meat and fish were largely introduced in their dietary. As to scurvy, this theory will be discussed in the following paper. As for ship-beri-beri Bullmore does not base his opinion on direct observations of food on board beri-beri-vessels. And though the Committee mentions seven crews who got bad provisions and were attacked by the malady, in none of these cases was it evident that the cause was damaged tinned articles of food. In some of these cases, indeed, the tainted aliment consisted in potatoes; but they were thrown overboard, and the disease did not break out before from 4—6 weeks afterwards. On the other side, the report of the Committee contains evidences of six crews being attacked in spite of the food being proved to be of good or even excellent quality. Finally it may be added, that the medical inspector of the Medical Government Board of Norway, Dr Geirsvold, Christiania, has examined a great number of boxes of tinned meat and fish from six beri-beri-ships. He examined them microscopically and made aerobic as well as anaerobic cultures of their contents; but none of the boxes showed any sign of being tainted. On the contrary, all boxes were sterile except one containing a small number of an aerobic bacillus, which had not been able to develop under the anaerobic conditions inside the tin.

It seems a more probable theory that the disease is due to some special form of underfeeding, *i.e.* that it is due to a food containing some, but not all necessary nutritive elements. Starting from the above-mentioned observation of Stian Erichsen concerning the connection between the disease and tinned food, it is, in the first place, a question whether the high temperature or steam-pressure used in the manufacture of such food, may not destroy some nutritive elements of the meat and fish. Many observations tend to show that strongly heated *milk* may cause another form of scurvy, *i.e.* *Barlow's disease*. And experiments in Java, described below, have proved that chickens get polyneuritis when fed on meat boiled for a long time at 120° C.

Tinned food may also be of importance in other respects. Such food in the long run is not palatable. The sailors, therefore, as a rule, eat little of it. The result is that their diet becomes comparatively

*one-sided, consisting chiefly of farinaceous constituents*; and these aliments are, to a large extent, not so good as the corresponding articles of food on shore. I may, in the first place, draw attention to the fact that the sailors on long voyages are very often reduced to *preserved, i.e. dried, instead of fresh potatoes*. That the dried potatoes often have something to do with the disease seems probable, because the disease often breaks out from 4—6 weeks after the fresh potatoes have been finished; and *vice versa* the patients often recover as soon as they get fresh potatoes on shore or from a passing vessel. This may be due to different causes. For instance the nutritive value of the potato may already be diminished by drying; neither dried potatoes nor dried green vegetables were found to protect against ordinary scurvy during the North American war of rebellion. But perhaps also the special methods of preparing the preserved potatoes may have an influence. They are not simply dried; if this is done they get an ill-coloured, greyish appearance. They are therefore first boiled in water containing hydrochloric acid or, sometimes, sulphate of lime, before being dried at 90° C.

It must further be mentioned that the Norwegian sailors on long voyages are, to a large extent, reduced to eating a *poor bread*. The government has, since 1895, ordered the sailors to use as much soft bread as possible. On long voyages they are, however, to a large extent compelled to use old flour, which does not bake well. At the same time ordinary bakers' yeast does not easily keep on long voyages in the tropics. Instead of yeast, which has been shown, in Germany, to be a remedy against scurvy, the sailors therefore very often use a farrago of fermenting squashed potatoes, groats, molasses, hop and other stuffs. The stewards are, in addition, not always good bakers, the result being, according to the papers of Nocht and Kjennerud, that the bread is to a large extent raw and not easily digested.

Finally, it may be mentioned that *beri-beri* has often appeared on Norwegian ships in spite of a daily use of lime-juice. But, it is true, this point has not been quite cleared up; the juice may, for instance, have been adulterated.

Considering these possibilities of an explanation of the disease, I have made a considerable number of experiments on animals. My starting point has been the excellent researches of the Dutch authors Eijkman and Grijns on the so-called *polyneuritis gallinarum*. Dr Grijns most obligingly showed me his experiments during my stay in

Batavia in 1902. About 10 years ago Eijkman<sup>1</sup> found that chickens get polyneuritis and die when fed only on *peeled rice*, i.e. rice-groats; the same happened when they were fed on sago or tapioca; in one case the same applied also to barley-groats<sup>2</sup>. On the contrary, the animals did not get ill after *unpeeled* rice, barley, oats or rye<sup>2</sup>, or after meat, boiled at 100° C.; nor did they get ill when Grijns<sup>3</sup> added small quantities of a special sort of beans (*Phaseolus radiatus*—in Malay, Katjang-Idjo) to the peeled rice. But if these beans were boiled for two hours at 120° C., Grijns stated that they lost their preventive power; the same applied, also, to meat and—to some extent—to unpeeled rice. Finally meat also produced the disease when boiled for some days at 100° C.

These experiments of Grijns have recently been continued by Eijkman, who found that unpeeled rye, oats, millet and barley produce the malady when boiled for two hours at 115, 125, and 135° C. respectively. As far as oats are concerned, this effect did not, however, appear after a boiling for two hours at 110° C. Nor did three chickens get any neuritis when fed on *horse-flesh*, boiled for two hours at 120° C.

There has been, in the Dutch medical press, a discussion, still going on, regarding the bearing of these experiments on the causes of tropical beri-beri. The more, because Vorderman found, in prisons of Java, a marked congruence between the frequency of the latter disease and the extent to which the prisoners were fed on peeled or “half-peeled” rice. One of the latest phases of this discussion has been the book of Hulshoff Pool<sup>4</sup> who believes that he has proved, by means of many observations on men, that *Phaseolus radiatus* protects also against tropical beri-beri. I do not propose to enter into this discussion; but it seemed to me that a continuation of the experiments of Eijkman and Grijns might, perhaps, throw some light upon the possible unwholesomeness of the articles of food which I have mentioned in connection with ship-beri-beri.

<sup>1</sup> *Virchow's Arch.* vol. CXLVIII. 1897.

<sup>2</sup> *Archiv für Hygiene*, vol. LVIII. 1906.

<sup>3</sup> *Geneeskundig Tijdschrift voor Nederlandsch Indië*, vol. XLI. 1901.

<sup>4</sup> *Beri-Beri. Voorkoming en Genezing door Toediening van Katjang-Idjo (Phaseolus radiatus)*, L. Amsterdam 1904).

*Experiments on pigeons.*

My first experiments were on pigeons. These animals are cheaper than chickens and do not take so much room; at the same time it has been shown, by Grijns, that pigeons, also, are susceptible to the polyneuritis.

The experiments gave the following results:—When fed exclusively on ordinary *rice-groats* and water, pigeons die without any exception after 3—7 weeks. This occurs whether the rice is raw or boiled for half-an-hour at 100 or 110—120° C. In some cases immediately, in others 8—14 days after the commencement of feeding, the animals begin to emaciate; and they die with a loss of weight of 25—50%, on an average 40%. Before death many of the animals seem paralytic; their gait is unsteady and stumbling, and they are not able to fly. But in other cases these symptoms are less prominent.

I have made 30 experiments with rice-groats. Post mortem there is as a rule a distinct but moderate oedema under the skin of the legs and feet, extending in few cases to a universal anasarca. Sometimes the oedema is only to be found on the upper limbs or on the throat and head; or there is no oedema at all. Sometimes there is also some hydro-pericardium and very seldom ascites.

When examined microscopically, the nerves show, to a varying extent, typical Wallerian degeneration, but without any other proliferation of cells than the multiplication of the nuclei of the sheaths of Schwann, which occurs in all cases of Wallerian degenerations; *i.e.* the process is no inflammation in the common sense of this word.

The number of degenerated fibres was usually greatest in the nerves of the lower limbs, where it was, in 15 of the 30 cases, very great. In the other 15 cases the corresponding number was moderate or small. Still, in these last cases I very often found some greatly degenerated fine twigs or single fibres between the muscles.

On the whole, the process is most pronounced in the smaller ramifications of the nerves, though this is not always distinct. The degeneration affects also the nerves of the skin; on the other hand, I have only exceptionally been able to prove its presence in the vagus nerve.—As to the spinal cord, there are usually some, but rarely many affected fibres in the white substance: in the grey substance I have, in accordance with Eijkman, not been able to prove any alteration of importance.—The muscles of the limbs and the heart often show fatty degeneration, but without any proliferation of cells.—It may be added,



that there sometimes appear small haemorrhages in the heart: haemorrhages rarely appear in other organs.

Proceeding to the question whether the disease may not be produced by other than tropical cereals, I made experiments with *barley*. In accordance with the corresponding experiments on chickens made by Eijkman, I found that unpeeled barley is an excellent food for pigeons. But when fed on *barley-groats* the animals die as constantly and after the same time as when fed on peeled rice. I have made 16 experiments with barley-groats. Seven of the animals showed a very great, nine but a moderate or small number of degenerated fibres in the nerves of the lower limbs. I further have tried with *barley flour*. Two animals died after being fed for 24 to 39 days on flour mixed with water and dried. Both of them had a very great number of degenerated fibres. Three animals received flour mixed with salt and water and baked as the so-called “flat-bread”—a hard, paper-like sort of bread, which is used in country places in Norway. The animals ate this food with avidity, but died after 35, 48 and 55 days, the first one with a great, the last ones with a moderate number of degenerated fibres in the nerves of the lower limbs.

As, however, Norwegian sailors do not eat much barley, I tried *rye-flour*, the effect of which has not hitherto been examined. I fed four animals on bread baked with yeast, while four got bread baked with “Royal baking powder.” This last bread is not very porous, has a viscous crumb, is often badly baked, *i.e.* to some extent raw, and so far corresponds with the bread which the Norwegian sailors commonly receive, as mentioned above, on long voyages.

For the rest, each sort of bread contained 8 grms. NaCl pr. kilo of fine-sifted flour. The result of these experiments was negative. Some of the animals lost weight at first; but after four months with one exception all of them were alive and apparently quite healthy. This animal had been fed on yeast-bread and acquired a somewhat stumbling gait: it therefore was killed. But no degenerated fibres could be found in the nerves. The muscles of the lower limbs, it is true, showed a moderate fatty degeneration; but this alteration I have found several times in pigeons kept in cages for months even when fed on good food (peas).

Taking a similar result for granted I have made no experiments with unpeeled rye. The Norwegian sailors do not, however, use much rye on voyages in the tropics, since they can get, in tropical ports, hardly



anything but wheat-flour. Nor has the effect of this nutriment yet been examined. I therefore have fed pigeons on wheat bread, baked with 8 grms. NaCl pr. kilo of flour. Again I fed some of the animals on bread baked with yeast, and others on bread baked with Royal baking powder. Like the corresponding rye-bread the latter was not very porous, partly raw, and with a viscous crumb.

Two series of experiments were made with these two sorts of bread, each series comprehending eight animals. The results of the first series were as follows: three of the four pigeons fed on bread prepared with baking powder, died after 30, 32 and 42 days, the two latter with a great, the first with a small number of degenerated nerve-fibres. The fourth animal as well as the four pigeons fed on yeast-bread were alive after three months; but all of them had lost about 30 % of their original weight. They were killed, and one of the animals, fed on yeast-bread, had a great, the others but a small number of degenerated fibres in the nerves of the lower limbs.

The results of the second series of experiments were about the same. The four animals fed on baking-powder-bread, died after 41, 43, 51 and 100 days, the others after 90, 103 and 116 days. Of both kinds two animals had a great, one a moderate and one but a small number of degenerated fibres in the nerves of the lower limbs.

*These experiments show that bread of wheat-flour, i.e. the flour ordinarily used by Norwegian sailors on tropical waters, is much more injurious to pigeons than rye-bread. The experiments further show that the effect of wheat-bread similar to the poor quality bread used by Norwegian sailors on long voyages, was far more injurious than that of bread baked with yeast.*

I may finally add that the latter difference cannot be ascribed to any poisonous effect of the baking-powder, because the rye-bread, prepared in the same way, did not give the same result.

As to *unpeeled wheat*, three pigeons were fed on this food for four months without any effect. The same applies to two animals fed for four months on *peeled oats*, the effect of which has not yet been examined either. The latter applies also to *oat-flour*, which was baked as the "flat-bread," mentioned above. This nutriment (mixed with water) produced, after three months, no effect on three pigeons. Taking the same result for granted, I have not made experiments with unpeeled oats.

Pigeons died constantly when fed on boiled potatoes, no matter whether fresh or the dried (*preserved*) potatoes mentioned above. The

effects of these two kinds of food were not wholly identical, since the fresh potatoes did not cause any marked polyneuritis except in one case out of 11. This pigeon did not die till the 153rd day. The others, however, showed very few degenerations of the nerves. One of these animals died after 80, the other nine after 18—39, or an average of 30 days. On the other hand, the dried potatoes produced an extensive degeneration of the nerves in three out of six cases: the other three pigeons showed a comparatively small number of degenerated fibres, though more than in the 10 fresh-potato-animals. These six pigeons lived for 45—60 days after the beginning of the experiment. It may, however, be objected, that the great majority of the fresh-potato-animals only lived a comparatively short time; had they lived longer, some of them might possibly have also got an extensive neuritis.

Considering that Norwegian sailors eat much fish, I also made experiments with so-called *fish-balls*, which food, when tinned, is much used on board ship. I added, however, much more flour to the fish than is generally used otherwise, preparing the balls of pounded codfish and wheat or potato-flour in equal proportions. The paste prepared in this way was boiled for 10 minutes and afterwards cut in small pieces and dried at 50° C. in order to preserve it. Before use the pieces were again soaked in water. I fed four animals on balls made of wheat-flour and four on balls made of potato-flour. The first ones died after 31—47, the last ones, which did not eat so well, after 9—43 days. Three of the first and two of the latter had a great many degenerated fibres in the peripheral nerves. In the remaining cases the number of degenerated fibres was but small. It may, it is true, be objected, that the fish, through being dried, may have lost in nutritive value.

The results of feeding with potatoes and potato-flour, mentioned above, differ from the experiments made by Eijkman with potato-flour without any addition of other food. His experiments (on chickens) gave a negative result, except when the flour was boiled for two hours at 125° C. Still, Grijns observed that chickens got polyneuritis, when exclusively fed on raw potato-flour. Personally I have not made corresponding investigations.

I further tried to ascertain what quantities of dried peas and unpeeled barley—both excellent food for pigeons—must be added to rice or barley-groats in order to prevent the development of polyneuritis. The result was, that a daily addition of 5 grams of peas or unpeeled barley was sufficient; while an addition of 1—2 grams in some cases had no preventive power, in other cases proved to be very advantageous. I have for instance fed one pigeon one year on 1 gram of peas a day in addition to barley-groats, the only result being that it lost somewhat in weight.

I also made experiments in order to ascertain whether neuritis may be produced by a simple starvation. The results will be mentioned below.

Finally I have studied the effects of *strongly heated food*. As to dried peas, this article of food seems to produce, when boiled for  $\frac{1}{2}$  hour at 120° C., a more pronounced moulting of feathers (four pigeons) than when eaten raw (four animals)

or boiled at 100° C. ( four animals). But otherwise all these animals seemed after 4 months to be healthy and none of them had lost in weight, nor did the former, when killed, show any degeneration of the nerves. The same applies to four pigeons fed on unpeeled barley, boiled for  $\frac{1}{2}$  hour at 120° C. compared with four fed on the same barley in the raw state; all these animals, too, were after 4 months in splendid condition, though the former had moulted a good deal more feathers than the latter. Nor did some animals get ill, that were daily fed on 5 grams of dried peas or 5 grams of unpeeled barley, boiled for  $\frac{1}{2}$  hour at 120° C. in addition to peeled rice or barley.

### *Experiments on chickens.*

Of quite another kind were the results of feeding on strongly heated *ox-beef*. Following Grijns I made experiments on chickens with this food.

As mentioned above Grijns<sup>1</sup> found that chickens get polyneuritis when fed on beef, boiled for two hours at 120° C. His experiments comprehended eight animals. One chicken did not get sick in spite of being fed during 11 months. As to four of the other animals, the result was negative, not very distinct, or doubtful, one dying without degenerations after 9 days, two with few degenerations after 19 days and 5 months, and one after 19 days with paralysis, but without its nerves being examined. The remaining three chickens died after 14 and 15 days and 5 months, all of these animals having a great number of degenerated fibres in their peripheral nerves. In these experiments, however, the beef was more strongly heated than in the manufacture of tinned meat. For instance, according to the paper of Bischoff and Wintgen<sup>2</sup>, the meat is boiled in a German manufactory for from 1 $\frac{1}{4}$ —2 hours at 100° C. and afterwards only for 1 hour at 120° C. The same period of boiling at 120° C. is also indicated in the *Calender für Conservenindustrie*, 1905. It may also be objected that the experiments of Grijns were made with meat from the Java ox ("Karboŭwen"); this meat does not taste quite as our beef does. Finally it may be added, that the corresponding experiments of Eijkman<sup>3</sup> gave negative results. He fed three chickens on *horse-flesh*, boiled for 2 hours at 120° C. one of the animals died after 1, another after 4 months; both had lost considerably in weight, but did not show any neuritis. The third animal had, after 4 months, lost in weight; but otherwise it was apparently healthy. I made two experiments, each on four chickens.

<sup>1</sup> *Geneeskundig Tijdschrift voor Nederlandsch. Indië*, vol. xli. 1901, No. 1, pp. 30—31.

<sup>2</sup> *Zeitschrift für Hygiene* 1900, vol. xxxiv.

<sup>3</sup> *Archiv für Hygiene*, 1906, vol. lviii.

In the first experiment two animals were fed on beef boiled for  $\frac{1}{2}$  hour at  $100^{\circ}$  and afterwards for 1 hour at  $120^{\circ}$  C., two control animals got the same beef, boiled for  $\frac{1}{2}$  hour at  $100^{\circ}$  C. All boiling was done in an ordinary autoclave; the beef was always minced and was found to be thoroughly boiled. The result is shown in the Table below. After 3—4 weeks the weight of all animals had increased. But afterwards their appetite diminished and their weight slowly decreased. At last the animals took very little food, became very emaciated, and died, or were killed, as shown in the Table. During the last 3—4 weeks all the animals showed a pronounced inclination to lie down.

At first sight it might appear that the result of this experiment was negative, *i.e.* that there was no difference between the two couples of chickens. The difference was, however, very marked. In the first place, both animals fed on 120 degrees beef, showed very pronounced oedema under the skin of the lower limbs as well as of the abdomen and throat. Both of them, also, had moderate hydropericardium. These alterations were, however, not marked in the two other animals, though the subcutaneous tissue of the one that lived longest, was somewhat watery. In the second place both animals fed on 120 degrees beef showed a *very extensive degeneration of the nerves of the lower as well as of the upper limbs*, the number of affected fibres being very great, both in the nerve trunks and in their finer ramifications. On the other hand, the radial nerve of the one that lived the shortest time of the controls, as well as the sciatic and peroneal nerves from the one that lived the longest time of the latter, contained but few degenerated fibres; most of them were to be found in the latter animal.

TABLE.

*Chickens fed on beef boiled at 100 and 120° C.*

	Weight of animals in grams											
	day 1st	day 7th	day 16th	day 23rd	day 30th	day 37th	day 44th	day 55th	day 65th	day 79th	day 89th	day 96th
100 degrees beef, 1st chicken	700	770	880	1000	1020	1000	930	940	935	885	—	865 killed
100 degrees beef, 2nd chicken	910	880	1020	1140	1135	1130	1100	1040	1040	800 died	—	—
120 degrees beef, 1st chicken	910	890	1080	1210	1220	1160	1120	1030	1030	840 killed	—	—
120 degrees beef, 2nd chicken	750	740	890	950	1000	890	900	870	870	720	570 died	—

Some few degenerated fibres often appear in the nerves taken from quite normal animals: this I can state from observations on guinea-pigs



and rabbits. Hence the difference between the two couples of chickens was in reality very marked.

In the second experiment two chickens were fed on beef boiled  $\frac{1}{2}$  hour at  $100^{\circ}$  C.: the other two were this time fed on the same meat, boiled for  $\frac{1}{2}$  hour at  $110^{\circ}$  C. The boiling was, as before, done in the autoclave, and the beef, which was cut in small pieces, always proved to be thoroughly boiled. This time all the animals ate well and increased in weight, until one of them, fed on 110 degrees beef, and having apparently been well, suddenly was found paralytic in the cage the 49th day after the beginning of the experiment. It died 5 days afterwards showing emaciation, some hydropericardium and somewhat watery subcutaneous tissue, but no distinct oedema. Microscopically, *the larger nerves as well as their finer ramifications were extremely degenerated*, both in the lower and upper limbs. In addition, the muscles of the extremities and the heart showed fatty degeneration. In the white substance of the spinal cord there were also some, though not many degenerated fibres.

The remaining three animals continued to increase in weight, until they were killed on the 68th day. None of them seemed ill, but the nerves of the second 110 degrees animal contained a moderate number of degenerated fibres in the larger trunks and their ramifications in both the lower and upper limbs. In the two 100 degrees animals only the sciatic nerves contained some few degenerated fibres. *It therefore seems evident, that beef may produce polyneuritis in chickens, even when boiled at a lower temperature than the  $120^{\circ}$  C. commonly employed in the manufacture of tinned meat.*

Both the chickens fed on 110 degrees beef increased less in weight than the other two. The day before one of them became paralytic it had only increased 515, and the other 525 grams since the start, against 835 and 775 grams of the controls. And when the remaining three animals were killed, the surviving chicken fed on 110 degrees beef had increased 865 grams since the start against 925 and 1100 grams for the controls.

The more strongly-heated beef produced therefore some underfeeding. This was—at least chiefly—due to the fact, that the animals did not eat this beef as readily as the less boiled one. This was evident from the quantities of beef eaten by each animal, which were this time weighed daily from the start. Adding together these quantities, the one 110 degrees animal had, the day before it got paralysed, altogether eaten 7685 and the other one 8980 grams; the corresponding figures with respect to the 100 degrees animals were 10,395 and 9210 grams. And when the three remaining animals were killed, the surviving 110 degrees animal had, since the start, altogether eaten 14,050 grams against 15,800 and 15,095 grams, for the two 100 degrees animals. But the figures given also show, that the difference was



comparatively small. *It therefore seems impossible to ascribe the polyneuritis to an underfeeding in the ordinary sense of this word.* This conclusion must also be drawn from the previous experiment. In connection with this subject it also may be mentioned, that Eijkman did not find any polyneuritis in chickens fed on so small quantities of unpeeled rice, that they died from starvation. Nor have I found any polyneuritis myself, experimenting in a similar way with pigeons. These animals, when left to themselves, eat 25—30 grams of dried peas or unpeeled barley daily. Instead of this quantity, I gave each of 12 pigeons 5—10 grams daily, with the result that they died after 5—7 weeks, but without any of them having polyneuritis. In spite of the pronounced emaciation and loss of weight mentioned above, which pigeons show when fed on peeled rice, barley, &c., and in spite of the loss of appetite, which I have repeatedly found accompanies these symptoms, *the polyneuritis cannot in these cases either be due to an underfeeding in the common sense of this expression.*

Before leaving the experiments on chickens, it may be added, that the beef always had been recently boiled. That is to say, the effect of the strongly-heated beef can only be ascribed to a decomposition of one or several constituents of the beef produced by the high temperature or high steam-pressure used in the boiling.

The foregoing experiments may in various directions need to be supplemented. For instance, I twice saw polyneuritis occur in poultry yards in Norway. The first time about 30 of 200 chickens died of paralysis. I only got one carcass for examination; this chicken showed a very extensive neuritis of the limbs. As to the food, I only know that the chickens chiefly, if not exclusively, had got coarsely ground Indian corn, which food, as far as I can judge from two experiments, does not produce neuritis in pigeons. As soon as the food on my advice was altered, the diseased chickens recovered, and the mortality ceased at once; but I do not know, in what direction the food was altered. The second time three chickens out of seven died, one after the other, with paralysis; before death their gait was unsteady and stumbling. I made the post mortem of one of them and found a very pronounced oedema under the skin of the throat, neck and head; there also was some hydropericardium. Microscopically, I found a moderate neuritis of all limbs. In this case, the animals were to a large extent fed on fresh potatoes, which food, as mentioned above, only produced a distinct neuritis in one out of 11 pigeons.

A further pursuit of the experiments of Eijkman and Grijns may also be of interest on account of the additional light they may throw upon the deterioration of food owing to strong heating. We have seen, that this is of importance in weighing the question of the nutritive value of tinned food. But the same applies also for instance to the steam-boiling of various articles of food, used in many modern hospitals.

This boiling is often done at an over-pressure of  $\frac{1}{2}$  atmosphere, *i.e.* a temperature of about  $110^{\circ}$  C., which boiling, when applied during  $\frac{1}{2}$  hour, produces, as we have seen, an alteration in the nutritive elements of beef.

Several chemical questions arise. For instance, which are, properly speaking, the nutritive constituents, the presence of which prevent, and conversely, the absence of which produce the disease? That such elements exist has been suggested by Grijns, and seems to be proved by Eijkman<sup>1</sup> who was able to cure the malady by adding to the injurious food an aqueous extract of rice-bran.

I have, however, for the present abstained from trying to answer these and other questions concerning *polyneuritis gallinarum*, because the experiments mentioned above have not thrown any clear light upon the question, which has been to me the principal one, *i.e.* the etiology of ship-beri-beri. It is true, the experiments described have shown that more of the ordinary articles of food produced *polyneuritis gallinarum*, than appears from the papers of Eijkman and Grijns. The experiments also support the suspicion, mentioned above, as to the injurious effects of tinned meat and poor quality bread, used on long voyages. On the other hand, however, the experiments have not demonstrated in any convincing way the injurious effect of dried potatoes, though the latter, to judge from facts, seem to be often connected with the malady. It may further be added, that *polyneuritis gallinarum* resembles tropical beri-beri much more than the ship-disease, neuritis being present much oftener than in ship-beri-beri. And finally, even if modified feeding of pigeons and chickens had produced a disease more like ship-beri-beri, the objection would still remain that we cannot, from experiments on poultry, draw any convincing conclusion concerning man.

I therefore discontinued the experiments on poultry and passed over to investigations on mammalia. These latter investigations have been carried out in conjunction with Dr Frölich, and some of the results will be discussed in the following paper.

<sup>1</sup> *Arch. f. Hyg.* 1906, vol. LVIII.

## EXPERIMENTAL STUDIES RELATING TO SHIP-BERI-BERI AND SCURVY.

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(Plates XVII and XVIII.)

### II. ON THE ETIOLOGY OF SCURVY.

- (1) *On the macroscopical alterations in the tissues of guinea-pigs which had been fed exclusively on bread, groats, and unpeeled grain.*

By experimenting with the "one-sided" diets which were used in the experiments, mentioned in the foregoing paper on polyneuritis in poultry, we found that guinea-pigs also contract a disease, and that this disease is accompanied by very characteristic changes.

These alterations however do not as a rule develop until from three to four weeks after the beginning of the experiment. Young animals, weighing less than 150—200 gms. could not be used because they died in one or two weeks, and although older guinea-pigs, especially at the beginning of our experiments, remained alive for a longer period, we frequently lost these animals within a fortnight. This happened especially when they were fed on ordinary rice. These rice-animals as well as all guinea-pigs fed on other sorts of grains which died within 14 days, will be briefly mentioned in the 3rd section of this article.

The present section of our paper deals with the 64 animals that died in 18 days or more when fed on ground or unground oats, barley, rye, or wheat and water. To these we add one animal fed on rye-bread that died in 15 days. The weight of the animals was usually 300—600 gms. Apart from two guinea-pigs fed on a mixture of oats and rye-bread, each animal received one single nutriment only.

We commence with the introductory information given in Table I.

TABLE I.

Nutriment	Effect on pigeons	Effect on guinea-pigs	Number of examined animals	Molar teeth		Number of animals the osseous system of which was examined microscopically
				Examined in number of animals	Found loose in number of animals	
Oats	0	Death after 22-28 days	4	4	4	4
Rye	Not examined	24-28 „	4	4	4	4
Wheat	0	25-29 „	4	4	4	4
Barley	0	26-46 „	7	3	3	3
Oaten groats	0	28-33 „	9	9	9	9
Barley groats	Death after on an average 5 weeks	21-41 „	13	2	2	2
Rye-bread baked with yeast	In some cases some loss of weight; but otherwise no effect after 4 months	15-46 „	10	6	6	9
Do. baked with baking-powder	Do.	32-37 „	4	0	0	3
Rye-bread baked with yeast, and oats	Not examined	27-29 „	2	2	2	2
Wheat-bread baked with yeast	Death after 3-3½ months	23-36 „	6	4	4	4
Do. baked with baking-powder	In most cases death after 30-51 days, in some cases after about 3 months	33 „	2	0	0	0
Total			65	36	36	44

This table shows, that whereas only barley groats and wheat bread—or in other words certain kinds of ground grain—were observed to have a marked effect on pigeons, *the results with guinea-pigs were quite otherwise*. These animals invariably died, no matter whether the grains were ground or not. In the 65 animals, death occurred, on an average, after 30 days.

During the experiment the animals became emaciated, the corresponding total loss of weight being, on an average, 40% of the weight at the beginning of the experiment. In some cases the weight decreased uniformly, but as a rule the animals at first lost weight, then either increased or remained unchanged for 3—14 days, and finally exhibited a definite decrease in weight during the week or so preceding death.

Moreover, these experiments afforded, in other respects, quite different results to those obtained with pigeons. In the first place it must be pointed out that the majority of the guinea-pigs exhibited, at the post-mortem, *pronounced haemorrhages*. These haemorrhages appeared most frequently in the muscles of the hind-limbs, where they sometimes infiltrated large portions of both thighs and legs. In other cases the

haemorrhages were found only in one thigh and one leg. In the legs they appeared as a rule in the upper part of the peroneal muscles from which they often spread to the fascia round the knee-joint. In the deeper layers they could often be traced to the periosteal tissue around the epiphyseal lines of the tibia and fibula. At the same time, there were frequently to be observed haemorrhages in other muscles, particularly in the intercostal muscles around more or less numerous junctions between the ribs and their cartilages. Sometimes all the ribs were affected in this manner, and some haemorrhages in this locality were almost constant. The same observation applies to haemorrhages in the deeper muscular layers and in the periosteal tissue at the inside of the lower jaw. Finally there appeared in several cases, haemorrhages in the muscles of the fore-limbs, of the back, and of the abdomen.

In addition to these muscular haemorrhages we observed often, although far less frequently, subcutaneous haemorrhages. These also appeared, as a rule, on the hind-limbs.

In all, we noted haemorrhages of the kinds mentioned in 51 of our 65 animals (about 80%). It is probable however that they occurred more often, because observations of those localities in which the haemorrhages appear, were not made with equal accuracy throughout the entire course of our experiments. In particular, we did not during the first period regularly seek for haemorrhages around the foremost ends of the ribs, and at the inside of the lower jaw. Yet subsequent investigations showed us that these localities are particularly instructive in this respect.

Below we further illustrate the occurrence of haemorrhages by means of some examples taken from the journal of our post-mortem examinations; but before doing so we desire to mention a number of additional points.

We observed in our animals petechiae in the skin. With one exception however (where they occurred in the skin of the abdomen) they were to be found only in the follicles of the vibrissae of the lips and muzzle. Here they seemed to be constant.

Small haemorrhages from the mucous membrane of the stomach were to be met with rather frequently. The same remark applies also to a diffuse injection—with or without bloody contents—of the small intestine, and also minute haemorrhages of its peritoneal cover. These peritoneal haemorrhages, in conjunction with a pronounced injection, were especially frequent in the duodenum. The latter in some cases



also showed ulcers in the mucous membrane, and occasionally these had perforated and caused a peritonitis.

We also in some cases noted haemorrhages in the kidneys, the liver, the spleen, and the lungs. The two first named had in other respects a normal appearance. The spleen was usually small. Sometimes the haemorrhages were so extensive that the liver, for instance, appeared excessively anaemic. This was especially so in the case of animals fed on oaten groats.

In addition to the haemorrhages there occurred a subcutaneous oedema. This however was not frequent, and was nearly always limited in extent. It appeared, for instance, in the paws, on one or even both thighs, in the axillae or on the lower jaw. In the experiments here described we only once saw a universal anasarca. In other cases where no subcutaneous oedema could be seen, we found some oedema of the muscles.

We further found at the post-mortem, a pronounced fragility of the bones. This remark applies in several instances to the tubular bones, and repeatedly when removed from the body the upper epiphyses of one or even both humeri or tibiae, or the lower epiphyses of one or both femora, separated from the corresponding shafts. Microscopic examination proved that this was due to fractures of the shafts just below the intermediate cartilages. As will be shown in the next section of this article, we have, in other cases where no macroscopical separations of this nature took place, found microscopically smaller or larger fissures passing horizontally through the shafts just below the epiphyseal lines, but without laceration of the periosteum: or there appeared microscopical fractures of the cortical substance. We have therefore concluded that the macroscopical separations between the epiphyses and the shafts existed *intra vitam*, but that the intact periosteum kept the fractured bones *in situ*. On several occasions we were able to prove the correctness of this view at the post-mortem, for after removing the periosteum it was sometimes easy to observe that the epiphyseal ends of the ribs were fractured and separated from their cartilages in correspondence with the haemorrhages in these localities as described above. [See the 4th animal, the post-mortem of which is described in detail below. See also some of the records of the animals in Table III, section 4.] In one instance we also found after a careful preparation of both tibiae, that the upper epiphyses of these bones lay movable on the top of the shafts, and were only connected with the latter by the periosteum.

When closely examined the surface of the tubular bones was found

to be rougher than is the case normally. This modification was especially remarkable at the surroundings of the epiphyseal lines. Here also the marrow shone through the cortical substance with a more reddish appearance than in the control animals. Sometimes we found holes, the size of a pin's head, in the cortical substance when the periosteum lay in direct contact with the marrow. The osseous *trabeculae* of the spongy substance of the long bones were, when compared with those of control animals, nearly always markedly rarefied. This was particularly noticeable when cutting through the upper ends of the tibiae.

A pronounced fragility was also repeatedly observed in the lower maxilla. In many cases the hind part of this bone fractured with remarkable ease when we attempted to remove it. Sometimes it actually crumbled between the fingers. In one instance the hind part of this bone was fractured *intra vitam* with a considerable amount of surrounding haemorrhage. Another animal showed fractures of both *rami ascendentes* just beneath the articulations. We also very often observed macroscopical defects of the osseous substance of the lower jaw. In one case its hind portion was perforated by numerous minute holes, but as a rule the defects appeared on the outer surface of the bone. The largest were the size of a hemp seed, and we further observed that in advanced cases the openings took a horse-shoe form. They corresponded to the root-tips of the molar teeth. We also frequently observed similarly situated defects in the upper maxilla. In this locality they were found at the base of the *antrum Highmori*.

Not only where these defects were pronounced, but in all cases examined in this direction, we found without a single exception more or less looseness of the molar teeth. Usually most of the molars were affected: more rarely only one or two were loose. In some instances they could with ease be removed by the fingers, and in pronounced cases after a careful preparation a gap between the teeth and the walls of their alveoli might be observed. In other words the latter had become too wide for the teeth.

On the other hand we have in only a few instances found the incisors loose. The reason probably is, that the incisors have long curved roots, and therefore do not so easily lose their hold.

Whilst discussing the teeth we may mention that they nearly always had a somewhat greenish-grey colour, whereas under normal conditions the teeth of guinea-pigs are pearly white. We also observed that in one case the pulp of a molar tooth, and in another instance

the pulp of both incisors, could be seen through the intact enamel as a dark red substance. Microscopical examination proved that this was due to diffuse bleeding in the pulp.

As regards the gums, these also often possessed a somewhat greenish-grey tinge. In 12—that is 18 %—of our 65 animals we noted that the gums were conspicuously hyperaemic and sometimes swollen. However this remark applies only to the gums on the front of the lower—in a few cases of the upper—incisors. Occasionally we found macroscopical haemorrhages under the mucous membrane of the gums. We have however never been able to detect ulcerations of the latter, nor could we see with the naked eye, any distinct traces of blood upon the surface of the mucous membrane. (As regards the microscopical observations, see the next section.)

Subjoined we give, in illustration of the observations just described, notes on four animals examined at different periods of our investigations.

1. Animal fed on *rye-bread* (baked with yeast) and water. Weight 1st day, 442 gms., died the 34th day, weight 220 gms. Diffuse haemorrhages in the peritoneal covering of duodenum, with hyperaemia of its mucous membrane. Bloody contents in the whole colon. Diffuse haemorrhages in the muscles of both thighs—particularly in the right—with a corresponding subcutaneous oedema; haemorrhages in the upper part of the peroneal muscles on both sides. A subcutaneous haemorrhage, the size of a pea, on the back. The gums on the front of the lower incisors somewhat hyperaemic. When removed, the upper epiphyses of both tibiae separated from their shafts, and diffuse haemorrhages were discovered in the corresponding periosteum and in the surrounding muscles. Ribs, inside of lower jaw and molar teeth not examined. (Microscopically, the upper end of the shaft of the right tibia shows very extensive haemorrhagic alterations of the specific kind described in the next section. Other bones were not examined in this way.)

2. Animal fed on *wheat-bread* (baked with yeast) and water. Weight the 1st day, 480 gms., died the 25th day, weight 285 gms. Small haemorrhages in the peroneal muscles of both sides and in the muscles of both antibrachiae. Enteritis, with bloody contents throughout small intestines, as well as in colon; petechiae in the walls of the caecum. No alterations in the stomach and duodenum. All lower molars, especially the rear ones, were loose and exhibited a somewhat greenish discoloration; the same applied to the back molars on each side of the upper jaw. Diffuse haemorrhages in the periosteum at the inside and outside of the latter bone, the cortical substance of which was very thin but without distinct defects. Ribs not examined. (Microscopically the marrow of the upper end of the shaft of the right tibia and of the lower end of the shaft of the right femur showed slight specific alterations. These, however, could not be detected in the upper end of the right humerus. Other bones not examined microscopically.)

3. Animal fed on water, and *oat groats* boiled for 15 minutes in water at 100° C. Weight the 1st day, 395 gms.; died the 29th day, weight 190 gms. Subcutaneous

and muscular haemorrhages in both legs; the lower jaw very fragile, fractured when removed; defects in its cortical substance corresponding to all the root-tips of the molar teeth. The latter, together with the incisors of both jaws all loose, and the bone somewhat discoloured. Haemorrhages between the periosteum and bone at the inside of both *rami horizontales* of the lower maxilla. Disseminated haemorrhages in the walls of the small intestines and coecum; the contents of the latter mixed with blood. Several small erosions with haemorrhages in the mucous membrane of the stomach. The lower epiphysis of one femur separated from the shaft when removed. Haemorrhages around some of the foremost ends of the ribs. (These were not examined with respect to separation from their cartilages, but showed microscopically pronounced specific alterations. So also did the upper ends of the shaft of the right tibia, and the lower end of the shaft of the right femur.)

4. Animal fed on *raw, unpeeled wheat and water*. Weight 1st day, 575 gms.; died the 26th day, weight 315 gms. Haemorrhages under the skin, in the muscles, and in the periosteum around the lower end of the left femur and the upper end of the left tibia. Diffuse subcutaneous haemorrhages on the left brachium. Small muscular haemorrhages disseminated around the lower end of the sternum. Haemorrhages in the tissue around the foremost ends of three of the right ribs; after removal of the periosteum two of them were found to be fractured and separated from their cartilages. All the lower molars on the right, and one of the back ones on the left side, were loose; so also was one back molar on each side of the upper jaw. The gums and all the teeth were somewhat greenish discoloured. No distinct hyperaemia of the gums. On the right outside of the lower jaw and at the base of *antrum Highmori*, there were some defects of the cortical substance corresponding to the root-tips of some of the molars. Diffuse subperiosteal haemorrhages at the inside of both *rami horizontales* of the lower jaw. Diffuse hyperaemia of the small intestines and stomach, also of the duodenum, where there were also some petechiae in the peritoneal cover. (Microscopic examination of the two ribs mentioned above revealed very pronounced specific alterations. The 3rd rib gave no results. Apart from the usual atrophy of the osseous tissue the microscopical examination of the right tibia, femur and humerus gave a negative result.)

It will be seen therefore that the principal points of the observations described above were:

- (1) *Haemorrhages*.
- (2) *Loose teeth*, connected in several cases with a marked hyperaemia of the gums on the front of the incisors, and also in some instances with macroscopical haemorrhages under the mucous membrane of the gums.
- (3) A certain *fragility of the bones*, sometimes connected with demonstrable *intra vitam* fractures at the ends of the shafts.

From these observations we were led to assume that the disease might possibly be *scurvy*. It is true that there are some differences between the latter, as usually described, and the malady of our guinea-pigs. For instance, we only noted a marked hyperaemia of the gums



in 18% of our animals. Yet in cases of human scurvy we do not always find affected gums. During the siege of Paris, for example, Lasègue and Legroux<sup>1</sup> failed to discover affected gums in one-fifth of their cases. Moreover, they point out that these patients did not show carious teeth, which are also never to be found in guinea-pigs. We may, in addition, quote the observations of Berthenson<sup>2</sup> concerning an epidemic of 225 cases in St Petersburg. Of these more than one half did not show any affection of the gums. We may also draw attention to the observations of W. Samson v. Himmelstiern<sup>3</sup>, who usually failed to find affected gums during the beginning of the epidemic. Also, considering the fact that our animals, on an average, died before the expiration of one month, it is of interest to observe that G. Samson v. Himmelstiern (Looser, *l.c.*) has noticed that patients suffering from scurvy did not get affections of the gums until a comparatively late stage of the malady. However, we shall see in the following section dealing with the microscopical alterations, that the gums were much oftener affected than appeared to be the case from the macroscopical examination.

With regard to the fragility of the bones, there is no essential difference between ordinary human scurvy and the disease we have described. Several of the older writers refer to this symptom. For instance, in *The disease of London or a new discovery of scurvy*, published in London, in 1675, we find that the author, Gideon Harvey, had seen scorbutic patients whose bones were so fragile that they were liable to fracture on quite slight injuries. Looser (*l.c.*), who has written an extremely interesting paper in proof of the identity of ordinary scurvy and Barlow's disease, also gives extracts from some of the earlier works on this subject. We subjoin, from this article, the following quotation from Hoffmann:

"In this disease (*i.e.* ordinary scurvy) all bones show a great fragility. It is astonishing to hear that patients suffering from scurvy have fractured an arm when lifting it in order to remove something, or a leg when walking, neither are observations wanting to show that such accidents have happened when the patients moved in their beds. Recently we observed, at Münster, a scorbutic patient who fractured his leg, by moving in his bed."

Looser also quotes some observations of the German anatomist Fries who found it extremely difficult to prepare the skeletons of

<sup>1</sup> *Archives générales de médecine*, 1871, II.

<sup>2</sup> *Deutsches Arch. f. klin. Medizin*, 1892, vol. XLIX.

<sup>3</sup> Quoted by Looser, in *Jahrbuch für Kinderheilkunde*, Dec. 1905.



individuals who had died from scurvy on account of the fragility of their bones. We may mention, further, the often quoted remarks of Poupart<sup>1</sup> who found at post-mortems of all scorbutic patients below the age of 18, separations of the epiphyses from the shafts. In some cases he also found fractures of the ribs at their connection with the cartilages. The same fractures have been particularly mentioned subsequently by numerous authors, who at the same time found the fractures surrounded by haemorrhages (Looser). Finally we may point out that Uskov<sup>2</sup> examined, in cases of ordinary scurvy, the foremost ends of the ribs. Here he found a very marked microscopical rarefaction of the solid osseous substance.

As regards these alterations, therefore, there is a marked similarity between the disease of our animals and ordinary scurvy. In one respect, however, there is a distinct difference between the two maladies. This concerns the frequency of haemorrhages.

In the first place it may be objected that, apart from the petechiae on the muzzles and lips, we observed haemorrhages in 80% only of our animals. This objection is however insignificant because in all epidemics of scurvy there occur cases in which the patients are said to suffer from a "taint of scurvy" but without visible haemorrhages. (As shown in the official medical reports from the North American war of rebellion these patients not infrequently die suddenly.)

In the second place the objection may be raised, that apart from the above-mentioned petechiae on the muzzles and lips only one of our animals showed some few haemorrhages in the skin itself. This obstacle is however removed by a comparison of the alterations seen in our animals with those, not of ordinary scurvy, but of *Barlow's disease*, that is to say, scurvy in young children. As demonstrated, first in the excellent paper of Barlow and afterwards by many other observers—for instance by the German pathologists quoted in the next section of this paper—this form of scurvy is accompanied by the same separations of shafts and epiphyses, and by the same fragility of the bones, as we have previously described. For instance, in this disease haemorrhages appear regularly around the foremost ends of the ribs, which after removal of the periosteum are found, to a large extent, to be separated from their cartilages. We may also observe that Barlow, and subsequently other observers, found a universal rarefaction of the bone substance. There are also affections of the gums relative to the

<sup>1</sup> Quoted by Lind, Looser, Netter (*Semaine médicale*, 1899) and others.

<sup>2</sup> *Centralbl. für die medic. Wissenschaften*, 1878, vol. xvi.

stage of dentition, and there are periosteal, muscular and subcutaneous haemorrhages. Yet in the skin itself, haemorrhages are comparatively rare in this "infantile scurvy." It is true that they were observed in 182 out of 353 cases collected by the American Pediatric Society<sup>1</sup>, but of the 31 cases which Barlow<sup>2</sup> had collected in 1883, only three showed such haemorrhages. (In two of these three cases they were limited to the eyelids.)

(2) *On histological changes in guinea-pigs fed on unpeeled grains, groats, bread and milk.*

We have examined the nerves of many of the 65 animals mentioned in the foregoing section of this article and have repeatedly found many degenerated fibres in some of the finer muscular or diaphragmatic ramifications. In contrast to the experiments on pigeons which one of us has described in the foregoing article, we have, however, been able to detect a pronounced polyneuritis in two animals only. (One of them had been fed on wheat-bread baked with yeast; the other animal had been fed on barley groats, boiled  $\frac{1}{2}$  hour at 100° C.) These observations may possibly correspond to the doubtful cases of neuritis, which, under the name of "acrodynie," have been described as occurring in some cases of human scurvy<sup>3</sup>. They may also possibly correspond to the neuritis occurring in some cases of ship-beri-beri<sup>4</sup>.

We have further, in very many cases, examined the *muscles* of the limbs. The fibres of these were to a large extent more slender than usual and many of them showed some fatty degeneration; at the same time a larger or smaller number of them were often dissolved into rows of irregular hyaline clots which did not always stain in the same way as the unaltered fibres. Between these clots there could be seen here

<sup>1</sup> *Medical Record*, 2nd July, 1898.

<sup>2</sup> *Medico-Chirurgical Transactions*, vol. XLVI., London, 1883.

<sup>3</sup> See the remarks concerning the epidemic of scurvy during the Crimean War in the foregoing article on polyneuritis in poultry. Such cases occurred also in the epidemic of scurvy in the siege of Paris, 1871. (Charpentier, *Étude sur le scorbut en générale, l'épidémie de 1871 en particulier*. Paris, Adrien Delahaye, 1871, pp. 59—62.)

<sup>4</sup> We may add, that very many of our animals showed, when examined microscopically, an extensive degeneration of the *axis-cylinders* of the nerves without any degeneration of the myeline sheaths. This latter phenomenon proves, that the degeneration of the axis-cylinders has occurred only a short time before death. The same degeneration also occurred frequently in the animals, which, as will be shown in the next section of this article, were fed on cabbage or fresh potatoes only. This degeneration, therefore, has no particular connection with scurvy.

and there small accumulations of the multiplied sarcolemma-cells; but otherwise no multiplication or immigration of cells could be discovered.

There was further in several, but not in all cases, a marked *fatty degeneration of the heart* as well as of the epithelium of the mucous membrane and the glands of the stomach and intestines.

In particular we have submitted *the osseous system* of our animals to an extensive microscopical examination, because numerous microscopical examinations have proved that *infantile scurvy* presents absolutely specific alterations of the bones. Before turning to speak of our own investigations on this subject we shall briefly sum up the results of these latter researches.

Thomas Barlow<sup>1</sup> in his classic treatise on infantile scurvy observes: "The periosteum of the femur vascular and thickened, but without cellular infiltration; extensive haemorrhage in the deeper portions and also between the periosteum and bone; considerable absorption of the trabecular structure with large spaces showing in places slightly eroded margins."

In 1890, Thomas Fischer<sup>2</sup> described a case in which he found that the bone-marrow at the ends of the shafts of the long bones directly beneath the intermediary cartilage had assumed an appearance like that of gelatinous tissue and contained abundant extravasations of blood. In these localities of the long bones there also was a pronounced absorption of the bone trabeculae. He also found similar changes, but even more pronounced, in the ribs.

Afterwards there appeared a series of communications from German pathologists on microscopic examinations of the osseous system in cases of infantile scurvy. For instance, Nägeli<sup>3</sup>, Jacobsthal<sup>4</sup> and Hoffmann<sup>5</sup>, published casuistic articles on this subject, to which Schödel and Nauwerck<sup>6</sup>, Schnorl<sup>7</sup> and Eugen Fränkel<sup>8</sup> drew attention by their

<sup>1</sup> *Medico-Chirurg. Transact.*, London, 1883, p. 187.

<sup>2</sup> *Münchener medic. Wochenschr.*, 1890, No. 36.

<sup>3</sup> *Centralbl. für allgem. Pathol. und pathol. Anatomie*, 1897, vol. viii. p. 687.

<sup>4</sup> *Ziegler's Beiträge zur pathol. Anat.*, vol. xxvii. p. 173.

<sup>5</sup> *Ibid.*, Supplement (*Festschr. für Professor Jul. Arnold*), 1905. Other isolated cases are described by Stoos and Butzke.

<sup>6</sup> *Untersuch. über die Möller-Barlow'sche Krankheit*. Jena, 1900.

<sup>7</sup> *Centralbl. f. allgem. Pathol. etc.*, 1899, vol. x. p. 834; *Ziegler's Beiträge*, 1901, vol. xxx.; *Jahrbuch für Kinderheilkunde*, January, 1907.

<sup>8</sup> *Fortschritte auf dem Gebiete der Röntgenstrahlen*, 1904, vol. vii. Nos. 5 & 6, 1906, vol. x. No. 1.

searching investigations on an extended scale. The number of published cases amounted recently to a total of not quite 30.

These investigations have given the uniform result—a result that constitutes a law—that infantile scurvy is accompanied by the following alterations:

(1) The *bone-marrow* loses, in quite definite localities, its lymphoid cells. These are replaced by a reticular or fibrillated, sometimes also a homogeneous, tissue containing only a few or no osteoblasts and marrow cells; instead of the latter there appear spindle-shaped or stellated cells. As regards the blood-vessels, their number, too, is considerably reduced. In this tissue there appear often, but not always, fresh haemorrhages or blood-pigment. Because this marrow is poor in cells it does not, for instance with haematoxylin-alum, stain as well as the normal one. In stained sections it can therefore often be distinguished from the normal marrow by its palish colour (“Helles Mark” of the Germans).

This reticular, fibrillated, or homogeneous tissue is considered by the German pathologists as the remaining unchanged or somewhat altered reticular ground or “supporting” substance of the original lymphoid marrow; they therefore often call it “frame-work” or “supporting marrow” (“Gerüst-” or “Stütz-Mark”; Schödel and Nauwerck).

These alterations appear in more or less numerous tubular bones or ribs, being limited, as mentioned above, to quite definite localities of the bones; for they are limited to the zones of the *endochondral ossification*, that is to the *ossification nuclei* of the *epiphyses*, but especially to the *ends of the diaphyses*. Here the “Gerüst-Mark” is intercalated as a layer of varying thickness between the intermediate cartilages or—as regards the ribs—between the cartilages of the latter and the normal marrow of the bone. Going downwards from the upper epiphysis of a tibia, we meet first the intermediary cartilage. Next follows a layer, “Helles Mark,” which often may be 1—1.5 cm. thick. Beneath the latter the marrow is again normal throughout the whole length of the bone until possibly a new layer, “Helles Mark,” may appear above the lower intermediary cartilage. (See Plate XVII.)

Finally it may be mentioned that the same diseased marrow is often to be found in the Haversian canals.

(2) There appears a *defective new formation of bone with a defective apposition on that already formed, which atrophies* and is absorbed. These alterations, too, appear principally though not exclusively, in the zones of ossifications, particularly in the ends of the shafts of the tubular bones and in the foremost ends of the ribs.



This atrophy of the bone-substance results in a pronounced rarefaction of the osseous trabeculae, while the remaining ones become slender or irregular, or appear, in microscopical sections, as isolated islets without any connection with the intermediate or—in the ribs—with the rib cartilages. With respect to the cortical substance, this too, becomes, in the same situation of the shafts, markedly reduced. Apart from macroscopical fractures, this reduction results occasionally in microscopical ones or in infractions; in places there is no cortical substance to be seen at all. Finally it may be added that the cortical substance in the ends of the shafts is often found perforated by comparatively numerous canals containing fibrillated tissue.

As the result of these alterations the bones become fragile, which fragility chiefly affects the ends of the shafts of the long bones just beneath the intermediate cartilages or—in the ribs—at their foremost ends. This is the reason why the so-called “separations of the epiphyses,” that is fractures of the shafts at their epiphyseal ends, so often occur in cases of infantile scurvy.

The above-mentioned alterations have already been shortly summed up in the first of the above-mentioned articles of Schmorl. He says, that the alterations of the bones are, in infantile scurvy, characterised by an affection consisting of a defective apposition and an increased absorption. That further the marrow in the ends of the shafts of the long bones and in the ossification nuclei in the epiphyses is deprived of its lymphoid cells, which are replaced by a fibrillated tissue, which is poor in cells, blood-vessels and osteoblasts. The correctness of these observations with the supplements described above have afterwards been confirmed from all quarters. At the same time, these affections are never to be found in any other diseases of children. It follows, “that infantile scurvy is an absolutely specific disease, a disease *sui generis*” (E. Fränkel).

(3) *Alterations of the intermediate cartilages and the cartilages of the ribs.* With respect to these, the authors have found the rows of the cartilaginous cells irregularly arranged and a pronounced persistence of the zone of calcification in form of a network of trabeculae of calcified cartilage.

The alterations mentioned above apply to infantile scurvy properly speaking. With respect to older scorbutic patients, there have, hitherto, only been published very few corresponding investigations. One of



them has been briefly described by Looser (*l.c.*). He examined the bones of a boy of 14 years, who died of sporadic scurvy, and succeeded in finding just the same alterations as those mentioned above. Another case has been very minutely described by E. Fränkel<sup>1</sup>. His examinations apply to a boy of 7 years, who also died of sporadic scurvy and who had been, for several months, fed almost solely on rice.

In the bones from this case, Fränkel found alterations which were absolutely identical with the affections of the osseous system in infantile scurvy; he therefore has no hesitation in declaring both diseases identical.

Being under the impression that perhaps Russian medical literature might contain publications bearing upon this subject we wrote to Professor Moissejew in St Petersburg and asked if he could give us some particulars.

He was kind enough to reply that Dr Krivoucha in St Petersburg had examined, in 1888, the bones from six cases of scurvy in adults, in which he had found: in the marrow of the tubular bones a network of fibres, the meshes of which were filled with lymphatic cells and red blood-corpuscles; in the ribs a reticular tissue, between the fibres of which there was shown lymphatic elements, red corpuscles and pigment-cells. Further he found absorption of the compact and spongy bone-substance. Though it does not appear whether these alterations were found exactly in the ends of the shafts and the ribs, their correspondence with those mentioned above is very conspicuous indeed. When we add the researches of Uskov (*l.c.*) referred to in the foregoing section, who also found, by microscopical examination of cases of ordinary scurvy, a marked absorption of the bone-substance, we therefore assume scurvy in children and adults to be accompanied by the same alterations of the osseous system.

Turning to speak of our own researches we shall briefly premise the following:

From the guinea-pigs examined we usually removed, from each animal, one or sometimes both tibiae, femora and humeri; we have also usually removed some ribs and one or both jaws with their gums. Sometimes we also examined the fibulae and scapulae. In several cases in the beginning of our examinations we only removed one tibia; or we only removed one tibia and one femur.

These bones were either completely decalcified by nitric acid or—

<sup>1</sup> *Fortschritte auf dem Gebiete der Röntgenstrahlen*, vol. x. 1906, No. 1.

usually—by trichloracetic acid; or they were partly decalcified by Müller's fluid (Pommer's method). This latter process has extensively been used by the German pathologists; chiefly because there has been, to begin with, some doubt as regards a connection between infantile scurvy and rickets, the osteoid substance of which is stained, when decalcified by Müller's fluid, by means of carmine. This doubt has, however, been dissipated, rickets being proved sometimes, but by no means always, to accompany infantile scurvy. For our purpose Pommer's method has been of comparatively little use, because our animals but very rarely showed any microscopical alterations which might suggest any formation of osteoid substance. The only advantage of the method was, that it permitted a closer study of alterations in the cartilages. On the other hand, this decalcification takes a very long time: in spite of the bones of guinea-pigs being small, they as a rule could not be cut before several months.

Of the bones so prepared we have made sections, which we have stained by means of Delafield's haematoxylin or haemalum and eosin; when prepared by Pommer's method, we also used the first named stains in conjunction with carmine.

The alterations which we have found in the so prepared bones recurred with small modifications in nearly all the 45 animals which we have examined in this direction. We therefore limit ourselves to a summary of the alterations which were, for instance, to be found in a longitudinal section of the upper end of a tibia from the diseased animals.

The shaft presents a normal marrow and a nearly normal cortical substance except in the vicinity of the intermediary cartilage. Here the compact substance becomes thin and atrophied or it is often traversed by larger or smaller irregular channels filled with a fibrillar tissue which may show haemorrhages. So far may this rarefaction of the corticalis go that it here and there may be completely wanting or there remain only some few fragments of bone substance or spiculae surrounded by fibrillar tissue and haemorrhages.

It is evident that the result of this atrophy must be that the bone becomes fragile just below the intermediary cartilage. That this is so, is also proved by the fact that there often appear small microscopical fractures with extravasations of blood. Or there appear *microscopical fissures* proceeding horizontally beneath the intermediary cartilage into the spongy substance of the bone.

As a rule, however, these latter fissures are only seen in the *spongy*

*substance*, being due to an atrophy of the bone trabeculae. As regards the latter, some of them are extant though to some extent atrophied and irregular. To a large extent, however, they appear as thin or irregular fragments or islets without connection with the intermediary cartilage. Finally it happens rather often that there are only few trabeculae to be seen in a comparatively great part of the section.

As regards the apposition of new-formed bone this does not seem to exist. We have, however, sometimes, but not often, seen a little new formation of osteoid substance being stained red by means of carmine in bones prepared by Pommer's method.

It is evident that this alteration, too, must contribute, to a large extent, to the result, that the bone becomes fragile just beneath the intermediary cartilage.

As regards the *marrow*, it is, in the middle of the bone, quite normal. But proceeding upwards in the section the lymphoid cells disappear or get markedly scarcer at a varying distance from the intermediary cartilage; this alteration occurs either at once or little by little. The cells are replaced either by more reticular or more fibrillated tissue containing only few or no osteoblasts and consisting of spindle-shaped or stellated cells, in the meshes of which may be seen some few remaining marrow cells and a few blood-vessels.

This tissue fills up all the cavities between the irregular bone trabeculae in the end of the shaft and may enter into the intermediary cartilage. In some cases it only appears in some sections, but not in others. Or we found it only beneath some but not beneath all portions of the intermediary cartilage. When this happened, we have repeatedly been surprised to see that the atrophy of the bone trabeculae did not always correspond to the degeneration of the marrow. Very atrophied trabeculae might appear in a normal marrow and *vice versa*; a very pronouncedly degenerated marrow might include apparently well-formed trabeculae.

However, in general, this degenerated marrow appears as a continual layer beneath the whole cartilage. As a rule, it extends 1—2 mm. downwards from the latter, differing, not only in stained sections, from the normal marrow by its palish colour.

In this tissue there usually appear multiple haemorrhages, which may be comparatively large. But we must expressly draw attention to the fact that these characteristic alterations of the marrow also occur without any extravasations of blood. In many animals we have seen, in this tissue, smaller or larger accumulations of a hyaline substance.

In the cases in which we have found fractures between the shaft and the epiphysis (separations of the epiphyses), the line of fracture has gone through this zone and consequently also through the corresponding atrophied part of the cortical substance.

As regards *the epiphyses*, they do not contain, in animals of the weight used in our experiments, any ossification nuclei. Being, on the whole, very strictly limited to the ossification zones, the above-mentioned alterations of the bone-marrow did not appear in the epiphyses except in one single animal fed on rye-bread; in this case there was a narrow, but marked strip on the upper side of the intermediary cartilage, where the normal marrow was replaced by a reticular tissue containing a considerably reduced number of lymphoid cells. Otherwise the compact as well as the trabecular bone-substance is, in the epiphyses too, somewhat, but not much, reduced.

There remains to be mentioned the essential alterations of the *intermediary cartilage*. This is as a rule markedly narrow. Its cells are of an unequal size and are to a large extent irregularly heaped up instead of being arranged in regular rows; where these latter exist, they very often may be seen diverging against the periphery of the cartilage. The zone of calcification is preserved, but we have often found the network of calcified trabeculae irregularly arranged or in some places wanting. In some cases the primary marrow cavities, containing fibrillar tissue, enter into the cartilage separating the rows of its cells from one another.

Finally we may observe that the periosteum surrounding the upper part of the shaft is, as a rule, thickened both in the outer fibrous and in the inner osteogenetic layer; in both layers there may be seen large extravasations of blood which may communicate through the corticalis with hæmorrhages in the marrow.

*Consequently these alterations are, in all essentials, wholly identical with those found in human scurvy.* We must especially draw attention to the fact, that the bone-marrow is affected, in guinea-pigs, in quite the same way and in quite the same localities as in the latter disease. That is: there is intercalated a more or less broad or narrow layer of the "Helles" or "Gerüst-Mark" of the Germans between the intermediary cartilage and the normal marrow of the shaft.

We have, as an example of the usual affections of our animals, described the alterations of the upper ends of the *tibiae*. Here they are nearly always very prominent indeed. If possible, they are still more pronounced in the ribs, which are, like the tibia, affected just at



the connection with their cartilages, that is in their epiphyseal ends. Also here we meet exactly the same alterations of the cartilages and of the periosteum as well as the same reduction of the compact and trabecular bone-substance in connection with a reticular or fibrillated tissue, which may contain haemorrhages or not, and which may form a more or less narrow or broad layer between the cartilage and the normal marrow. Usually the most affected ribs are those surrounded by macroscopic haemorrhages. But this is not always so, some ribs being altered without the latter and *vice versa*: some ribs are surrounded by extravasations of blood without distinct alterations of the marrow. The reasons seem to be, that the surrounding haemorrhages of the periosteum and muscular tissue are due to small fractures of the compact substance which may occur before the alterations of the marrow have become manifest; and *vice versa*: the alterations of the marrow may be comparatively far advanced without the compact substance being correspondingly reduced.

Before leaving the ribs, we may add, that their cartilages are, on microscopical examination, often found to be to some extent driven into the marrow substance, while the adjacent cortical substance has become somewhat convex outwardly. Probably it is the act of respiration, which, in some way, wedges the rigid cartilage into the softened bone.

The same alterations which are to be seen in sections of the upper ends of the tibiae, occur also to a large extent in the lower ends of the shafts of the femora as well as in the upper ends of the shafts of the humeri. Also in these localities there appear the above-mentioned alterations of the solid bone-substance, of the periosteum, and the intermediary cartilages; and at the same time there appear, between the latter and the normal marrow of the shafts, more or less narrow or broad layers of a reticular or fibrillar tissue, which tissue may or may not contain haemorrhages. In these bones, however, the alterations are not so extensive and not so constant as in the ribs or tibiae.

How often these alterations occur, will appear from Table II.

This table shows, that the microscopical examination of the bone-marrow from only three of our 44 animals gave a wholly negative result. (Two of these guinea-pigs were fed on rye-bread baked with yeast or baking-powder. They belonged to the first period of our researches; in each case we only examined one tibia. However, with respect to the third animal, fed on oatens groats, the negative results applied to two ribs, one tibia, one femur, and one humerus.) On the other hand, the



TABLE II.

*Summary of the typical scorbutic changes of the bone-marrow comprehending all 44 examined animals.*

				The Ribs			Tibia			Femur			Humerus		
				Examined bones	Typical changes in	Normal marrow in	Examined bones	Typical changes in	Normal marrow in	Examined bones	Typical changes in	Normal marrow in	Examined bones	Typical changes in	Normal marrow in
Died after															
Oats	...	...	22 days	5	5		2	2		2	2		2	2	
			25 "	5	5		2	2		2	2		1	1	
			28 "	3	3		1	1		1		1	1		1
			28 "	3	3		2	2		2		2	1	1	
Rye	...	...	25 "	4	4		1		1	1		1			
			28 "	3	3		2		2	1		1	1		1
			25 "	2	2		1	1		1	1		1	1	
			24 "	2	2		1	1		1	1				
Wheat	...	...	27 "	1	1		1	1		1	1		1	1	
			26 "	3	2	1	1		1	1		1			
			29 "	3	3		1	1		1	1		1	1	
			25 "	2	1	1?	1	1		1	1		1	1	
Barley	...	...	26 "	3	3		1	1		1	1		1	1	
			27 "				1	1					1	1	
			26 "	4	3	1									
Oaten groats	...		29 "	2	2		1	1		1	1				
			31 "	2	2		1	1		1	1				
			29 "	2	2		1	1		1	1				
			33 "				1	1		1	1				
			29 "	2	2		1	1		1	1				
			28 "	2	2		1	1		1	?				
			31 "	2	2		1	1		1	1				
			29 "	2		2	1	1		1	1				
			24 "	2		2	1		1	1		1	1		1
									1						
Barley groats	...		29 "				1	1					1	1	
			29 "				1		1				1	1	
Rye-bread baked with yeast			21 "				1	1		1	1				
			31 "				1	1		1	1		1		1
			31 "	2	2		1	1		1	1				
			34 "				1	1							
			34 "				1	1							
			46 "				1		1						
			31 "	1	1		1		1		1		1		1
			33 "	2	2		1	1		1		1			
Do. baked with baking-powder			15 "	6	3	3	1	1		1		1	1		1
			34 "				1	1							
			37 "				1	1							
			33 "				1		1						
Wheat-bread baked with yeast			31 "	2	2		1		1	1		1			
			32 "	2	2		1		1		1				
			25 "				1	1		1	1				
			23 "				1	1		1		1	1		1
Rye-bread and oats	...		29 "	3	3		1	1		1	1		1		
			27 "	4	4		1	1		1		1	1		1

examination of the remaining 41 animals gave a positive result. Still it must be admitted that the specific alterations of the marrow did by no means always appear in all examined bones; for instance, they sometimes were only demonstrated in the ribs. The ribs were examined in 29 of the 41 cases, in 28 cases with a positive result. Tibia, femur and humerus were examined in 40, 33 and 18 cases respectively; in 33, 20 and 11 cases respectively the result was positive.

There remain some remarks concerning the microscopical examination of the *gums*. We have examined sections of the gums from 11 animals, fed on all sorts of the nutriments mentioned above, and have always found haemorrhages in the mucous membrane between some of the molar teeth. We have further, by microscopical examination, repeatedly observed blood in the *free spaces* between the molars; in some cases this blood could be seen to have percolated from the periosteum of the alveolar cavity. Very commonly there also existed more or less extensive haemorrhages in the periosteum and bone-substance at the walls and base of the alveoli. In connection with the atrophy of the osseous walls of the latter, these haemorrhages may be of some importance as regards the loosening of the teeth.

As already mentioned in the foregoing section, we have also, in some cases, found large haemorrhages in the pulp cavities of the teeth.

Finally we must draw attention to the interesting researches of Bartenstein<sup>1</sup>, who fed quite young guinea-pigs on both uncooked and boiled milk and produced, in both cases, a malady, which he considers as identical with infantile scurvy.

We are in some doubt as to the existence of this identity. It is true, his animals showed fractures of the tubular bones with surrounding haemorrhages; at the same time, there appeared an atrophy of the solid bone-substance which was apparently even more pronounced than in our animals. However, it may be objected, that the fractures are not expressly said to have been localised at the ends of the shafts; and apart from the surroundings of the fractures there did not appear any haemorrhages. At the same time, the communication of Bartenstein leaves some doubt with respect to the localisation and nature of the alterations of the bone-marrow, that he, too, was able to demonstrate. Finally we may add, that he did not examine the teeth.

We have therefore, to a rather large extent, repeated these interesting researches, and we have closely followed the *modus operandi* described by Bartenstein. As for our results, we limit ourselves meanwhile to the following general remarks:

Whether our animals received fresh or boiled milk, they showed, to a large extent, fractures; but these were often localised at the centres of the shafts. Further they were, in several cases, connected with a very pronounced formation of

<sup>1</sup> *Jahrbuch für Kinderheilkunde*, Vol. Lxi. 1905, p. 6.

callus. Though it must be admitted that we also have seen veritable "separations" of the epiphyses, the first named fractures are not analogous either with those of the animals mentioned above or with those of human scurvy. Nor did we observe haemorrhages apart from the fractures; this applies also to the ribs. With respect to the *teeth*, we have only seen a slight indication of looseness in one case. Finally it must be admitted, that the bone-marrow in the ends of the shafts and ribs was, in our animals, too, markedly altered, being poor in cells and often infiltrated by blood. However, this alteration was often accompanied by a diffuse degeneration of the lymphoid cells, which also appeared in the centres of the shafts, where the lymphoid cells, in cases of scurvy as well as in the guinea-pigs mentioned above, appear normal. This *diffuse* alteration manifests itself by the fact that the nuclei of the lymphoid cells cannot be stained distinctly.

We may add, that the altered tissue in the ends of the shafts and ribs had not the same distinct fibrillated or reticular character as the corresponding marrow in the guinea-pigs mentioned above. On the whole, it seems probable that this interesting disease of Bartenstein must, for the present, be placed in a class by itself.

- (3) *On the alterations seen in guinea-pigs which die of starvation or of the effects of feeding on cabbage, on fresh or dried potatoes, or on hay.*

We have seen in the foregoing section that guinea-pigs fed on different sorts of unpeeled grains, groats or bread also get the same microscopical alterations of the bones which are found, by numerous German pathologists, to be the essential alteration in Barlow's disease and which do not, according to the same authors, occur in any other malady of children. We have also seen that these alterations have been demonstrated in the few cases of scurvy in individuals of a more advanced age who have been more closely examined in this direction.

In the present section of our article we shall deal with experiments regarding the question whether these alterations are due to the injurious effect of some special nutriment or not.

In the first place we have tried to ascertain whether the malady described instead of being due to a direct effect of the food is caused simply by *starvation*. Because, as mentioned above, the animals, at their death, are found to have lost about 40% of their original weight, and because they do not eat much for about the last week of their life. Therefore, as a control, we gave two animals water only, while three others received daily 40—60 gms. of cabbage each (otherwise they eat, when fed on cabbage only, between 100 and 200 gms. a day).

The first-named animals died after a few days, the last ones after

10—12 days. At the microscopical examination the lymphoid cells of the marrow of the tubular bones were partially normal. In other bones, however, their number had greatly and equally decreased not only in the ends of the shafts but also and to the same degree in their central parts as well as in the epiphyses, that is in the localities which are, as we have seen in the foregoing section, not affected either in Barlow's disease or in the guinea-pigs described above. In "starvation-marrow," which we will, for the sake of brevity, call this affection, the remaining groups of lymphoid cells are separated by a mucous tissue without any haemorrhages and without fibres or reticular tissue. This affection corresponds with the "gelatinous" marrow which is so often found in human cases where death is due to wasting diseases.

As regards other symptoms, there were also in these animals some petechiae in the follicles of the vibrissae of the muzzle and lips. But otherwise there were no haemorrhages at all; at the same time, not a single tooth was found loose. Finally all animals showed a pronounced universal anasarca.

These alterations might have been anticipated. In the first place, the same "starvation-marrow" has already been described in starved animals by Neumann. In the second place, there does not appear any symptom of scurvy in *human* diseases causing an ordinary starvation. For instance, a cancer of the oesophagus or stomach does not give rise to any scurvy; on the other hand, such cases often show marked oedema.

We may add that a "starvation-marrow" without any haemorrhages was also often demonstrable in the young and other animals which we have seen, in the first section, to die within a couple of weeks when fed on unground grains, groats or bread. These animals very often were seen to eat very little or nearly nothing. The same applies to three of the six animals which we have fed on ordinary rice-groats. These animals died within 8—12 days. However, in the three remaining cases we found the same alterations of the marrow that are characteristic of scurvy. With respect to one of these animals, living for eight days, we found these alterations in two ribs and in the upper end of the right tibia and the lower end of the corresponding femur. In the two remaining cases, living for 8 and 22 days respectively, in which we only examined the right tibia, the result was positive. (We may add that the longest living animal showed loose molar teeth. But in no case were there macroscopical haemorrhages.)

We see, therefore, that *rice produces the same effects as the other kinds of grain* used in the foregoing experiments.



In the *second place*, we have successively fed 11 animals on *cabbage* only, eight of them died after 2—6 months, with a loss of weight corresponding to about 30—40 % of the original one. Also in these cases there appeared petechiae on the muzzles and lips. But otherwise there were no haemorrhages at all; at the same time the teeth were never loose and always shining and white. The marrow of the ribs was usually normal: to some extent, however, it also showed a slight indication of a starvation-marrow without any of the qualities described in the foregoing section. A starvation-marrow without any of these qualities was also generally to be found in the tibia, femur and humerus. In addition, there was some atrophy of the osseous substance of the bones which was probably due to the general under-feeding. Also in all these eight animals there was a marked subcutaneous oedema all over the body.

In the *third place*, we have fed 11 animals on *boiled fresh potatoes* only. All these guinea-pigs died after 2—5 months with the usual petechiae in the follicles of the vibrissae; as in the foregoing animals, there were no haemorrhages anywhere else, and all teeth were tight and shining white. The marrow of the ribs was usually normal. The same applied in some cases to the humerus, femur and tibia, while these bones, in some cases also some ribs, showed a more or less pronounced starvation-marrow without any trace of the ordinary scorbutic alterations. Sometimes there was a slight subcutaneous oedema; in other cases no oedema could be discovered.

It is thus evident that scurvy *cannot* be caused in guinea-pigs either by simple starvation or by diets of *any* kind; *on the contrary, the disease originates in these animals as well as in man as a result only of certain special diet.*

The correctness of this view is further supported by the fact, that dried potatoes produce scurvy. We have seen, in the foregoing article, that the experiments on *pigeons* did not show any convincing difference between fresh and dried potatoes. Not so as regards guinea-pigs. We fed ten animals on the latter. The potatoes, bought from a ship-chandler, were first soaked, and afterwards boiled for half an hour at 100° C. Six of the guinea-pigs died within a fortnight without particular alterations. The remaining four animals died after 15, 20, 22 and 26 days, the first one without, the three latter with loose molar teeth. At the same time, all four animals showed haemorrhages round some of the foremost ends of the ribs, which were shown microscopically



to be affected in the usual scorbutic way. In the longest living animal there also appeared extensive haemorrhages in the muscles of the thighs and of the axillae: there also was some hyperaemia of the gums in front of the lower incisors. Though we did not find any specific alteration of the marrow of tibiae, femora or humeri, these results support the working hypothesis, discussed by one of us in the foregoing article, that dried potatoes may be of importance in respect of the etiology of ship-beri-beri.

Finally, we also fed seven animals on hay and water alone. They died after about one month after developing what may perhaps be termed pseudo-scurvy. The alterations were as follows. The animals showed the usual petechiae on the muzzles and lips. But further four of them had one or two molar teeth somewhat loose. Two of them had also a little reddish-blueness of the gums on the front of the incisors of the lower jaw; finally two of them had small subcutaneous haemorrhages on the fore-limbs, while one animal showed a haemorrhage of the area of about 1 centimeter in the muscles of the right thigh. Other haemorrhages could not be discovered. In spite of these results the marrow of all bones examined, the ribs included, did not show any indication of a scorbutic affection; on the contrary, it was, in all animals, the most pronounced starvation-marrow we have seen. Even in the marrow of the ribs, which was, in the "cabbage-" and "potato-animals" quite normal, the lymphoid cells often were reduced, all over the bones, to an astonishing minimum. It may be added, that all seven animals had a marked, some of them a very pronounced, universal subcutaneous oedema; in some cases there was also ascites.

(4) *On the effects of certain so-called antiscorbutic nutriments  
and of certain salts added to bread or oats.*

In order to obtain further evidence as to whether the disease described in the two first sections of this article is identical with human scurvy or not, we have tried to find our bearings as regards a possible preventive effect of some of the nutriments known as "anti-scorbutics" from human experience. For this purpose we fed several animals on oats, bread or rice mixed with fresh apples, fresh potatoes, fresh lemon-juice or cabbage. Finally, we also for reasons mentioned below, examined the effects of an addition of carbonate of lime, in some cases also of a mixture of this salt and neutral phosphate of lime.

TABLE III.

(+ indicates a positive, - a negative result of the examination.)

Nutriment	Life-time in days	Loose molar teeth	Haemorrhages		
			Around the epiphyseal ends of the ribs	In the muscles, periosteum and fasciae of the limbs and trunk	In or under the skin
<i>Rye-bread</i> (prepared with yeast) and 30 gms. <i>fresh apples</i> per animal a day.	52	+	-	-	-
	39	(faint indication)	-	-	-
	...	Alive the 97th day	...	...	...
Do. 5 parts and <i>fresh boiled potatoes</i> 4 parts.	51	+	-	-	-
	23	(faint indication)	-	-	-
Do. and <i>fresh boiled potatoes</i> equal parts.	36	+	-	-	-
	63	(faint indication)	+	-	+
	48	(faint indication)	(faint indication)	-	(1 petechia in the skin of the right leg) + do.
Do. and juice of $\frac{1}{8}$ , from the 29th day of $\frac{1}{4}$ <i>lemon</i> per animal a day.	34	-	- <sup>1</sup>	-	-
	34	+	-	-	-
	30	(faint indication)	-	-	-
	30	+	-	+	-
<i>Unpeeled oats</i> and <i>lemon-juice</i> as above.	28	+	-	(in the muscles of the right leg)	-
	48	+	+ <sup>2</sup>	-	+
<i>Wheat-bread</i> , prepared with yeast, and juice of $\frac{1}{8}$ <i>lemon</i> a day.	19	-	-	-	(under the skin of both groins) +
	43	(faint indication)	-	-	(in the skin of the right fore-limb) +
Do. and juice of $\frac{1}{2}$ <i>lemon</i> a day.	29	-	-	-	(several haemorrh. in the skin on the chest and abdomen)
	85	+	-	-	-
Do. and 10 gms. <i>fresh cabbage</i> per animal a day.	46	-	+	-	-
	113	-	-	-	-
	109	-	-	-	-
	43	(abscess)	-	-	-
Do. and 15 gms. do. per animal a day.	8	-	-	-	-
	33	(pneumonia)	-	-	-
	105	-	-	-	-
	72	-	-	-	-
<i>Rice-groats</i> and 10 gms. <i>fresh cabbage</i> per animal a day.	40	+	-	+	-
	28	+	-	-	-
	61	(faint indication)	-	-	-

<sup>1</sup> In spite of the missing haemorrhages around the ribs, the foremost end of one of the latter was very pale and found after removal of the periosteum to be separated from the cartilage. Microscopically, the marrow of this rib was typically affected.

<sup>2</sup> Haemorrhages around the foremost ends of 2 ribs; after removal of the periosteum both found to be separated from their cartilages.



The results of the first named experiments may be seen from Table III. In connection with the latter we beg to remark as follows:

We have, hitherto, as a rule limited ourselves to comparatively large quantities of the antiscorbutic nutriments. We daily gave each guinea-pig juice from one-ninth to one-half lemon or 30 gms. of fresh apples in addition to oats or rye- and wheat-bread. (The weight of our lemons varied from about 120—150 gms., each giving about 35—45 c.c. juice. As for the apples, we used so-called "American" apples, each weighing about 100—120 gms.) A similar remark applies to a mixture of bread and fresh potatoes containing these nutriments in the proportion of 5 : 4 or 1 : 1. However with respect to the *cabbage*, we consider a daily quantity of 10—15 gms. as a very moderate quantity indeed.

In spite of these quantities it will be seen that an addition of *lemon-juice* did *not* prolong the life of the nine animals<sup>1</sup> fed on oats, rye- or wheat-bread. The same applies to three of the four animals fed on fresh apples and rye-bread and to three of the four animals fed on fresh potatoes and rye-bread. However, one animal of each of the two latter experimental series lived markedly longer than usual; and with respect to the quantities of fresh cabbage, mentioned above, their addition to wheat-bread or rice saved the lives of several of the 12 animals, that were fed on these nutriments, for a comparatively very long time.

*Far more pronounced was the effect of the antiscorbutic nutriments on the specific scorbutic affections. This effect was very conspicuous indeed.* For the table shows, that some of the animals did not present any indication of scurvy; in fact the macroscopical indications of this disease were so slight, that the examination had to be made very closely in order to detect them.

To begin with the teeth, they were not pronouncedly loose except in the three animals fed on oats and limejuice. They were, apart from the animals fed on oats, always shining white.

The table further shows that haemorrhages around the foremost ends of the ribs, so common after feeding on bread or oats, appeared in four only of 25 animals that got the same food with an addition of antiscorbutic nutriments. Haemorrhages in the muscles of the limbs and trunk did not appear except in two cases. And though it may be admitted, that haemorrhages in or under the skin were somewhat more frequent, *the bone-marrow of the great majority of the ribs examined and of all tubular bones examined was wholly normal.*

<sup>1</sup> Six other animals, fed on the same quantities of lemon-juice in addition to the same nutriments, died within 8 to 14 days.



Otherwise it appears from the table that several of the animals presented a more or less pronounced subcutaneous oedema, sometimes also an indication of ascites. In some cases, we found ascites only. Having comparatively seldom observed oedema or ascites in animals fed on oats, bread, etc., without any addition, we have imagined *the abortive scurvy* of guinea-pigs to be analogous to ship-beri-beri. This opinion perhaps scarcely agrees, however, with the following experiments made in order to ascertain *the effects of strong heating on the anti-scorbutic power of cabbage*.

These experiments concern nine guinea-pigs, all fed on the same wheat-bread, baked with yeast. Each of three of them received daily an addition of 30 gms. of cabbage, boiled for half an hour at 110° C.; each of three others received the same quantity of cabbage, boiled for half an hour at 100° C., whereas each of the three remaining animals obtained 30 gms. of the same cabbage unboiled. (The quantities of cabbage were always weighed in fresh state.)

The details of these experiments were as follows:

1. *Wheat-bread and 30 gms. of cabbage boiled for half an hour at 110° C.*  
*1st animal.* The weight on the 1st day was 415 gms. and kept nearly unaltered until the 25th day (400 gms.), when it began to decrease. (Died the 47th day (260 gms.)) A slight subcutaneous and considerable muscular oedema in the limbs and a slight subcutaneous oedema of the lower jaw were found. No haemorrhages; but the ribs were not examined. Some hyperaemia of the small intestines; all teeth tight. Microscopically the marrow of two ribs and of the right tibia and femur showed typical scorbutic alterations.—*2nd animal.* The weight being the 1st day 325 gms., increased slowly to 480 gms. (the 83rd day); thereupon it decreased slowly until the *death the 105th day* (325 gms.). Slight muscular oedema. Subcutaneous haemorrhages in the left popliteal and on the left knee. Haemorrhages round the foremost ends of several ribs. Hyperaemia without haemorrhages of the small intestines. The molar teeth loose. Urine very alkaline. *Microscopically* several ribs and the right tibia showed *typical scorbutic alterations*.—*3rd animal.* Weight at the start 440 gms.; increased slowly until the 94th day (525 gms.); thereupon it decreased little by little until *death the 125th day* (355 gms.). No oedema. Extensive haemorrhages under the skin, in the fascia and muscles of both hind limbs and round the foremost ends of several ribs. Molar teeth loose. Defects in the cortical substance on the outside of the lower jaw. Hyperaemia with haemorrhages of duodenum. Urine slightly acid. *Microscopically typical scorbutic alterations of the marrow* of several ribs, and of the right femur and tibia were found.

2. *Wheat-bread and 30 gms. cabbage boiled for half an hour at 100° C.* The three animals weighed at the start 300, 400 and 440 gms. respectively. They were killed the 153rd day weighing 440, 700 and 550 gms. respectively. They were macro- as well as microscopically *quite normal*<sup>1</sup>.

<sup>1</sup> We always examined several ribs as well as one tibia and femur.



3. *Wheat-bread and 30 gms. of fresh cabbage.* The three animals weighed at the start 350, 350 and 345 gms. respectively. *The 1st animal* increased slowly in weight until the 268th day (690 gms.); it is still living, weighing the 299th day 565 gms.—*The 2nd animal* was killed the 153rd day weighing 540 gms. It was macro- as well as microscopically quite normal<sup>1</sup>.—*The 3rd animal* was killed the 105th day as a control for the 1st 110 degrees animal; its weight was 330 gms. We found some subcutaneous and muscular oedema of the hind limbs. But otherwise all was normal; no haemorrhages; all teeth tight; microscopically the marrow of two ribs and of the right tibia, femur and humerus was normal.

Though we did not, in these experiments, observe the extensive oedema that we anticipated, the results present a new proof of the correctness of the view discussed in the paper on polyneuritis in poultry: *that there may exist a connection between strongly heated nutriments and scurvy.* However, at the same time, it must be pointed out that the preventive power of the cabbage had by no means been wholly destroyed by the strong heating. For two of the animals, fed on cabbage heated to 110 degrees, lived for a comparatively very long time; and with respect to the third animal, it died with tight teeth. That is, in spite of the strong heating of the cabbage, the animals were far better situated than was the case when they received no cabbage at all.

There remain to be considered some experiments regarding the question whether possibly the malady described in the first sections of this paper may be due to some injurious effect of the *acid salts* which are contained in grains, groats and flour.

The German author Weiske<sup>2</sup> has found that *rabbits* decrease in weight and die with rarefactions of the osseous substance when fed on *oats* only. He is of the opinion that this effect is due to the acid salts in connection with a want of lime. For an addition of carbonate of lime wholly neutralises the effects of the oats, while neutral phosphate of lime, though advantageous, has a less pronounced effect. Still less pronounced, though advantageous, is an addition of carbonate of magnesia.

These experiments have been repeated by Stoelzner<sup>3</sup>, who found the same alterations microscopically. If he fed rabbits on oats only, he found a pronounced rarefaction of the bone-substance with a defective or wholly wanting apposition of new-formed bone. If, however, he fed them on oats with *carbonate of lime*, the apposition was very pronounced; finally, if he replaced the latter salt by carbonate of

<sup>1</sup> We always examined several ribs as well as one tibia and femur.

<sup>2</sup> *Zeitschr. f. Biol.* 1895, Vol. xxxi.

<sup>3</sup> *Virchow's Archiv.* Vol. cXLVII. p. 430.

sodium, the result was somewhat less pronounced than when the animals received oats only.

We have attached the more importance to these experiments because not only oats but also the other one-sided diets on which we have fed our scorbutic animals are also poor in lime.

We have also taken into consideration the opinion of A. E. Wright<sup>1</sup>, who basing on the fact that grains, flour and some other nutriments contain acid salts, theoretically drew the conclusion that the rarefaction of the osseous tissue and the other symptoms connected with human scurvy are due to an intoxication by acids. He further ascertained a diminution of the alkalinity of the blood in seven cases of scurvy.

Though these latter results disagree with the researches made by Lamb<sup>2</sup> in 11 cases of scurvy, we have given 28 guinea-pigs liberal quantities of carbonate of lime in addition to the various sorts of grains, bread, etc., that were used in the experiments mentioned in the first and second sections of this article. This salt was used because it gave, in Weiske's as well as in Stoeckner's experiments, the best results. Each animal received some grams daily. However, the result was wholly *negative*. Nor were the animals protected by a mixture of carbonate and phosphate of lime.

At the post-mortem, we also examined, in 17 of the 28 animals, the reaction of the urine. In seven cases it was alkaline, sometimes strongly alkaline; in five cases it was neutral, and only in five acid. Though these results did not seem to agree with the hypothesis of an intoxication by acids; and though we further have found that the ashes of *dried potatoes*—which nutriment, too, produces scurvy in guinea-pigs—have a strongly *alkaline* reaction; we shall not, at present, draw any definite conclusion concerning the value of the acid-theory.

(5) *On etiological analogies between the scurvy of guinea-pigs and human scurvy.*

It remains for us to examine whether human scurvy can be caused by food similar to that which we have mentioned in the foregoing experiments.

Before entering on this question it may be useful to consider the present theories on the etiology of scurvy in general.

Of these theories there are three. The first one supposes the malady to be of an infectious nature. This idea, as shown in the work of Lind,

<sup>1</sup> *Lancet*, 1900, II.

<sup>2</sup> *Ibid.* 1902, I.

was held by Boerhaave; in the 19th century it was supported by Villemain; and in our days it is accepted by many Russian physicians. As far as we have been able to see, this theory is only based on speculation. Indeed, we have only seen the following two observations quoted in favour of the theory. The first one concerns an epidemic of "mild scurvy," which broke out in the German prison of Möhringen and its neighbourhood and was described by Kühn<sup>1</sup>. The disease attacked 253 individuals and seems to have been contagious; only 10 of the patients, however, showed haemorrhages of the skin, while many others had an urticaria, erythema or herpes. At the same time, not only stomatitis but also angina and bronchitis; that is a *status catarrhalis*, as well as intermittent chills and rheumatism, were frequent symptoms. No doubt, this disease was not ordinary scurvy but a *peliosis rheumatica* or similar undefinable malady. The second observation was published by Tschudakoff<sup>2</sup>. During an epidemic of scurvy in a Russian famine-district he observed, in connection with another Russian physician, 12 cases of sore gums in individuals whose food is said to have been "satisfactory." He therefore believes them to have been contaminated by the surrounding scorbutic population. No importance can, however, be attached to these cases for the reason that the author does not mention the special nutriment of which the "satisfactory" food consisted.

The *second theory* supposes scurvy to be due to *damaged food*. This theory can be seen, in the work of Lind, to date back to the middle ages; and there are still many authors who are of this opinion. Above all, many writers consider *damaged* salt meat to have been, for instance on board ships on long voyages, the real cause of many epidemics of scurvy. As will be shown below, we do not wholly deny every importance of such sorts of food. We must, however, point out that their influence has not been proved by any convincing observation. In fact, the theory is based on experiences during famines, campaigns, sieges, or on board ships on long voyages. That is under circumstances where it must be admitted, that various articles of food often are damaged. On such occasions, however, the selection of nutriment will also be very limited; that is the diet will become one-sided. How easily this will happen may be seen when comparing the article on scurvy of

<sup>1</sup> Über leichte Scorbutformen. *Deutsches Archiv. für klin. Medicin*, Vol. xxv. 1880, p. 115.

<sup>2</sup> Über das Auftreten des Scorbut in Zusammenhange mit Hungersnot. Inaugural Dissertation. Berlin, 1903, p. 27 a foll.

Immermann<sup>1</sup> with that of Hirsch<sup>2</sup>. The first one quotes two epidemics of scurvy on board the "Columbia" and among some gold-diggers following on damaged food; the last one points out that there had also, in these same epidemics, been a complete lack of fresh vegetables. The same applies to an epidemic among the Circassian cavalry during the Crimean war, quoted by W. Koch<sup>3</sup>. He tells that the troopers got some damaged fat and biscuits, in some cases also a little damaged peas, beans or mutton; but beyond this their food only consisted of one or two handfuls of rice daily. He further mentions an epidemic of scurvy in a French prison, published by Beumann<sup>4</sup>. Koch points out that the prisoners got damaged meat. In the original paper, however, prominence is given to the fact that, during the last two months before the appearance of the malady, the prisoners were deprived of fresh potatoes and that in addition to the meat, their food consisted only of dried beans, rice and similar nutriment.

Jackson and Harley<sup>5</sup> have supported the theory of damaged food by experiments on monkeys. When fed daily on tainted tinned meat together with some rice and Indian corn these animals got diarrhoea which was accompanied by bloody and mucous stools and spongy and easily bleeding gums. In some cases the gums showed also small ulcers. As far as we are able to see these experiments, however, only prove that tainted meat, when eaten daily, may provoke an intense inflammation<sup>6</sup> of the upper as well as of the lower part of the intestinal canal. If the experiments were also to throw light upon the etiology of scurvy, they ought also, at least in some animals, to have provoked haemorrhages in other organs than the alimentary canal; and even as regards the latter, the authors do not mention whether they found much or only very little blood.

Before leaving this theory we may, however, admit, that we do not deny all injurious effect from damaged meat or other damaged nutriment. In the first place such nutriment may, as shown in Jackson's and Harley's experiments, provoke an intense diarrhoea, which evil seems, for instance during the Crimean war, very often to have created

<sup>1</sup> Ziemssens, *Handbuch der spec. Path. und Therapie*, Vol. xiii. 1876, p. 560.

<sup>2</sup> *Handbuch der historisch-geogr. Pathol.* 1883, pp. 385—387.

<sup>3</sup> *Die Bluterkrankheit*, Stuttgart, 1889 (Billroth-Lückes Deutsche Chirurgie).

<sup>4</sup> *Archives générales de médecine*, 1887, i. p. 27.

<sup>5</sup> *Lancet*, 1900, i. p. 1184.

<sup>6</sup> Also six control monkeys got a fatal diarrhoea; but there did not appear blood or mucus in the stools; nor did the animals get spongy gums.



a predisposition to scurvy. In the second place, many people do not like to eat damaged nutriments. For instance, if the salt meat gets damaged on board a ship it may be imagined to cause scurvy, not because the sailors eat it, but because they do *not* eat it or because they eat too little of it and prefer to stick to the other, *i.e.* mostly farinaceous nutriments.

The *third theory* supposes the disease to be caused by a one-sided diet, especially a diet which does not contain, or contains too little of fresh aliments. It seems to us that the facts by which Lind, Hirsch and many other writers have supported this theory, are in every respect convincing, and they agree with our own experiments as to the influence of fresh cabbage, apples, potatoes, and so on.

It lies beyond the plan of the present article to enter into the details of these epidemiological observations. We may, however, draw attention to the fact that *scurvy has repeatedly arisen where the food consisted of the same or about the same nutriments as we have used in our experiments on guinea-pigs*. To commence with *infantile scurvy*, Barlow<sup>1</sup> has pointed out that this disease seems often due to the injurious effect of farinaceous food. In this connection he quotes Cheadle, who has been aware of the same coincidence, and who has raised the question why nearly all of the children who principally live on *bread*, escape scurvy. This question Cheadle has replied to in the same way, as we have, ourselves, so often done in respect of large parts of the Norwegian population, living chiefly on "bread and coffee." The answer is: because they live, at the same time, on potatoes.

As regards older individuals, a German author<sup>2</sup> mentions two women who got scurvy having been for several weeks reduced by poverty to a diet of tea and bread without sugar and milk. (See also one of Bucquoy's cases below.) Furthermore the Danish author Adolph Meyer<sup>3</sup> mentions a boy of 10 years who got the disease after having lived chiefly on cocoa and wheat-bread; he never ate vegetables, eggs, milk, butter, or fat, and had "without doubt but seldom" got any meat. Meyer also quotes a boy of five years, observed by Evans, who got the disease after having chiefly lived on bread and butter [?] and "a small quantity" of milk, while he never ate vegetables, meat or soups. We may further mention that cases of scurvy, of which some were fatal, were observed in con-

<sup>1</sup> *Medico-Chirurg. Transact.* 1883.

<sup>2</sup> *Encyclopädisches Wörterbuch der medic. Wissensch.*, von Busch, Dieffenbach and others. Berlin 1843, Vol. xxxi. p. 382.

<sup>3</sup> *Barlow's disease*, Copenhagen, p. 1901, p. 122 a.f.



nection with the so-called thirst-cures of Schroth, in fashion in Germany in the middle of the 19th century. In addition to a very small quantity of fluids, the food consisted, during these cures, of wheat-bread ("Semmel-Kur") and some porridge of rice and millet<sup>1</sup>.

Again, we may recall the interesting observations of Curran<sup>2</sup> during the epidemic of scurvy, which broke out after the failure of the potato crop in Ireland 1846-47. Four-fifths of the numerous patients concerning whom he got information had lived only upon bread and coffee or tea. The food of the remaining one-fifth had consisted of cereals of various kinds or cereals and flesh or fish; but in no single instance could green vegetables or potatoes be discovered to have formed a part of their regular diet.

It seems to us that such observations are convincing. In famines, flour and bread are very often the only foods on which the population can fall back. In this connection we may also draw attention to the following cases observed in Finnmarken, the most northern part of Norway, where the population is very poor, and sporadic cases of scurvy are not uncommon, especially during the failures of the potato-crop. These cases have been observed by Dr Wessel, Medical Officer of Health of Syd-Varanger, who has kindly permitted us to mention them.

1. A woman, 37 years old, came under observation 2/3/1907. Had been ill for five to six weeks; large haemorrhages of the skin of one leg; sore and bleeding gums. Had not got potatoes, fish or milk during the whole winter: no meat since the beginning of January. Had only lived on rye-bread, soup of oat flour and coffee without sugar.

2. A man of 44 years and his three children of four, six and eight years, came under observation 2/3/1907. The man had only sore gums, the others also extensive purpura. Two of the children had been ill since Christmas, one for from two to three weeks. Since October no potatoes or milk; they had lived on coffee, bread and a soup made of flour; very seldom meat or fish.

3. During the same epidemic a woman got haemorrhages and sore gums, having lived, for the whole winter, on flour and groats. Besides she had very little meat and no potatoes or milk.

4. Finally Dr Wessel mentions a patient who got, in 1901, sore gums and haemorrhages of the skin all over the body after living for a long time, exclusively on dry bread and black coffee.

Finally we may take the opportunity of pointing out that *rice, which is so often supposed to be the cause of tropical beri-beri,*

<sup>1</sup> H. Salomon, *Über Durstkuren*, Berlin, Hirschwald, 1905, p. 2; Jürgensen, *Deutsches Arch. für klin. Med.* 1866, 1.

<sup>2</sup> *Dublin Quarterly Journal of medic. Science*, August, 1877, p. 109.

*occasionally has provoked scurvy.* For instance, Delpech<sup>1</sup> mentions, in his article on scurvy in Paris in 1870-71, a patient who got the disease who for four months had only eaten meat four times, besides this, he had had nothing but rice. During the same epidemic Bucquoy<sup>2</sup> observed two cases of the same kind. The first patient had for three or four months lived exclusively upon rice and water thrice a day. The second patient had, for the same time, lived *almost* only on rice; in addition he had two or three times got beans or potatoes. A third and a fourth patient had, for the same time, only eaten bread and rice; a fifth patient only bread, rice and some few times meat; a sixth patient had only eaten bread. Again, we may quote the above-mentioned 10th of Fränkel's<sup>3</sup> cases, a boy of seven years with all symptoms of an ordinary scurvy, whose bones showed the regular microscopical alterations of Barlow's disease which we have observed in our guinea-pigs. Because he could not endure any other food, he had for months lived on rice only. Finally we may refer to the interesting article in which Maitland<sup>4</sup> has replied to the above-mentioned experiments of Jackson and Harley. He instances as one of the causes originating scurvy among sepoys the following: a sepoy wishes to save money; he therefore only eats the rice and dhal from the military kitchen and refuses to have such extras as fresh vegetables, meat or milk, because he is obliged to pay for them out of his own pocket. Within from 2-3 months, says Maitland, this man will get scurvy and will recover after a short time as soon as he gets fresh potatoes or lemons in addition to his previous food.

It is true, in this case the food did not exclusively consist of one single kind of grain. On the whole, it must be admitted that this happens rather seldom, and that most cases of scurvy have occurred after a somewhat more varied food. But in these cases, too, the food can often be proved to have been very like the nutriments which cause the disease described in guinea-pigs. For instance, each prisoner of the Akershus prison in Christiania, from the beginning of 1845, received 2 oz. of pork and 6 oz. of meat a week; once a week the prisoners got butter. Besides this, the food consisted of potatoes, milk, bread, broth, porridge of barley groats and barley flour, ale and a soup made of ale. Owing to the failure of the potato-crop, however, no potatoes were distributed from the middle of 1846; from this time, also, the prisoners

<sup>1</sup> *Annales d'hygiène* publ. 1871, Vol. xxxv.

<sup>2</sup> *Union médicale*, September and October, 1871.

<sup>3</sup> *Fortschritte auf dem Gebiete der Röntgenstrahlen*, 1906, Vol. x, No 1.

<sup>4</sup> *Lancet*, 1900, II. p. 1164.

only exceptionally got any milk. The result was, that after some months, there broke out a very extensive epidemic of scurvy<sup>1</sup>. This epidemic did not cease until, in 1847, fresh potatoes were again distributed.

We may further mention an epidemic in the Wartenburg prison in Germany<sup>2</sup>, where the food consisted of a soup of flour, porridge, bread, and different sorts of beans. Only twice a year was meat distributed, and owing to failure of the crop neither potatoes nor other fresh vegetables could be procured. Finally the prisoners were provided with fresh vegetables and got in addition meat several times a week; thereupon the epidemic rapidly disappeared. Again, Delpech (*l.c.*) records a patient, who got scurvy after for two or three months living only on bread, rice, soup and sometimes boiled peas. Finally it may be mentioned that the epidemics of scurvy which prevailed, during the siege of Paris, in several prisons of the city, began some time after the potatoes and other fresh vegetables had been finished and the food had been reduced to rice, bread and dried peas and beans (haricots) in addition to some wine and coffee with sugar; the beans and peas were partly replaced by maccaroni or barley-groats, boiled with a little fat<sup>3</sup>. It may be added, that the prisoners are expressly said not to have got any fresh, salt or smoke-cured meat.

Thus also epidemiological facts speak in favour of the opinion that the described disease in guinea-pigs is identical with human scurvy.

Before leaving this subject, we may, however, shortly draw attention to one objection, that possibly may seem obvious. This refers to the fact, that prisoners when being punished with "bread and water" (with some salt) are not known to get scurvy. However, in Norway the prisoners are not confined to this diet for more than 20 days (in Finland up to 28 days). As will be seen from the observations mentioned above, it takes, however, months before a bread diet produces scurvy.

#### CONCLUSIONS.

We have seen in the foregoing sections of this article, that a one-sided diet consisting of various sorts of grain, groats and bread, produces

<sup>1</sup> *Forhandlinger i Kristiania medicinske selskab.* (Transact. of the medic. Soc. of Christiania), *Norsk Magazin for Lægevidenskaben* (Norweg. Magaz. for medic. Science), 1847, p. 523.

<sup>2</sup> Wald, *Vierteljahrschrift für gerichtliche Medicin*, 1857, Vol. xi. p. 45.

<sup>3</sup> See the articles of Delpech (*l.c.*) and of Lasègue and Legroux (*Archives générales de médecine*, 1871, II.).

in guinea-pigs a disease that corresponds, macro- as well as microscopically, to human scurvy.

On the other hand, we have found that this disease does not occur after a one-sided diet consisting of fresh cabbage or fresh potatoes, whereas it again is produced by dried potatoes. That is, the disease originates in guinea-pigs as well as in man as a result of a diet confined to some special nutriment.

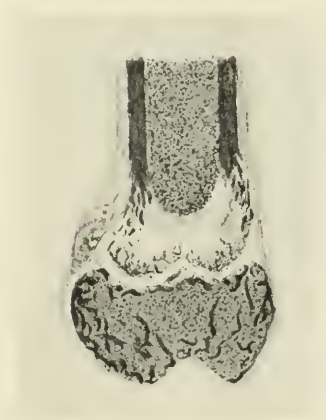
We have further observed that the disease is favourably influenced by different sorts of nutriment known, from human experience, as "antiscorbutics." We have, however, also found that at least one of these nutriment, that is cabbage, loses a deal but not all of its preventive power when boiled for half an hour at 110° C.

Finally we have quoted several examples showing that the same or similar one-sided diets that produce the disease in guinea-pigs, have repeatedly produced scurvy in man.

However, we have not, hitherto, been able to produce the disease that has been the proper aim of our experiments, that is the younger brother of scurvy or ship-beri-beri. It is true, we have repeatedly, in guinea-pigs, seen abortive cases of scurvy recalling the latter disease. This problem is, however, by no means clear. Nor have we, hitherto, been able to make experiments explaining, in an unmistakeable way, why the one-sided diets, mentioned above, produce scurvy.

*Postscriptum.* We had already finished the present paper when we examined two guinea-pigs that had been fed on wheat-bread and one that had been fed on rye-bread. In spite of their dying 29, 30 and 27 days after the beginning of the feeding, the microscopical examination of 2—4 ribs as well as of one tibia, femur and humerus from each animal did not show any scorbutic alteration. We are of the opinion, that this negative result may possibly have something to do with the fact, that the weight of the animals was 1140, 1085 and 995 gms. respectively; that is, the animals were comparatively very old. For we have pointed out, that the specific alterations of the bone-marrow are limited to the zones of ossification. In old animals, however, the ossification has, in all essentials, ceased. In one of the cases there were some muscular haemorrhages; all three animals showed discoloured and loose molar teeth.

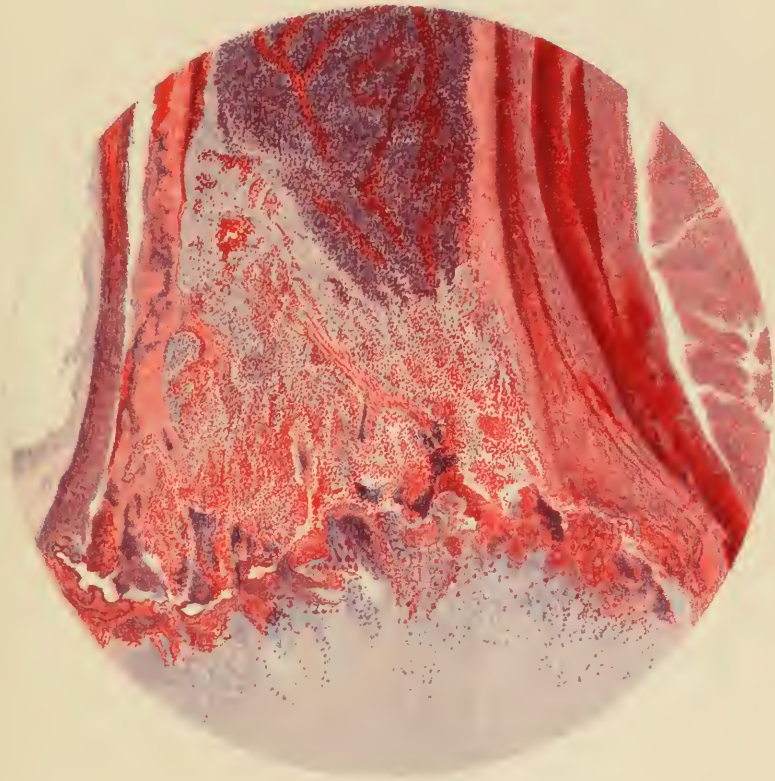
On the other hand, we arrived at other results when examining a dog, concerning which we got the following information:



Drawing showing a slightly enlarged section of the lower end of a femur from a guinea-pig which died after feeding on oaten groats. A pronounced layer of "palish marrow" between the intermediate cartilage and the normal marrow of the shaft. The bone trabeculae of the latter are very atrophied; the same applies to the corresponding part of the cortical substance. The section was stained with haematoxylin but is drawn with Chinese ink.







The figure shows a longitudinal section of a rib with the adjacent cartilage from a guinea-pig fed on rye-bread. Trichloracetic acid; haematoxylin, eosin. Only some of the cells of the cartilage are shown in the drawing. Small fissures between the cartilage and cortical substance as well as the bone-marrow. A broad layer of blood infiltrated "palish marrow" with few and irregular bone-trabeculae between the cartilage and the normal marrow. On the one side there are haemorrhages in the periosteum and also between the latter and the cortical substance. Leitz obj. 3; drawing-prisms.



It was fed, for two months after birth, on its mother's milk; thereupon it got, for 3—4 months, a mixed diet. About 5—6 months after birth it was, in addition to a number of other dogs, placed in a kennel and fed on oaten-flour, boiled with water, on some days also on Indian corn; at the same time the animals received a certain amount of mesentery of an ox boiled with water, but without the fat.

Fed on these aliments, the dogs began little by little to become weaker until the owner after 2—3 months changed the food for dog-biscuits. As soon as this was done, the animals recovered very rapidly. However, a month afterwards the owner reverted to the oaten-flour, etc. Again the animals became weak. After some months they therefore again received dog-cakes and recovered rapidly, whereas one dog was killed because of an apparent paresis of the fore-limbs. We did not get any nerves for examination; nor do we know anything certain with respect to haemorrhages or looseness of the teeth. However, we examined four ribs and the upper end of the right tibia as well as the lower end of the corresponding femur. *In all these bones we found microscopically a thick layer of typical reticular or fibrillated tissue with or without haemorrhages between the intermediary cartilage or rib-cartilage and the normal marrow of the shaft or the rib.* As for the solid bone-substance, it did not look very much atrophied; considering the very reduced or wholly wanting osteoblasts, we may, however, conclude, that the solid substance, too, was somewhat abnormal.

SOME OBSERVATIONS ON THE CONDITION OF THE  
BLOOD IN MEN ENGAGED IN ANILIN DYEING AND  
THE MANUFACTURE OF NITRO-BENZINE AND ITS  
COMPOUNDS.

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CONTENTS.

I.	Preliminary . . . . .	672
II.	Anilin dyers . . . . .	673
III.	Conclusions concerning Anilin . . . . .	676
IV.	Nitro-benzine workers . . . . .	676
V.	Conclusions concerning Nitro-benzine . . . . .	679
VI.	Animal experiments . . . . .	679
VII.	Conclusions from animal experiments . . . . .	683
VIII.	General Summary and Conclusions . . . . .	684

I. *Preliminary.*

It has long been known that men engaged in Anilin dyeing and in the manufacture of Nitro-benzine frequently suffer from various symptoms of poisoning. In the majority of cases the symptoms are slight and are not sufficiently severe to prevent the men from working; in some cases men are obliged to leave their work for longer or shorter periods. In a few cases death has occurred from the severity of the poisoning.

At the request of the Home Office Committee on Industrial Diseases, the towns of Bradford and Huddersfield were visited in January 1907 in order that information might be obtained as to the condition of the blood of the men engaged in Anilin black dyeing in the former and the manufacture of Nitro-benzine and its derivatives in the latter.

The following points were observed in all the cases examined :

The man's age, length of employment, number of times off work from illness due to trade; general appearance as indicated by cyanosis, and



conjunctival pallor. Examination of the blood included spectrum analysis, estimation of specific gravity, counts of both red and white corpuscles per c.mm., differential percentage count of the different kinds of leucocytes by means of stained films, and examination of the physical condition of the red corpuscles by the same means.

## II. *Anilin Dyers.*

The men examined were selected on account of their being engaged in those processes which seemed most likely to lead to poisoning; in fact two of the cases were men who were off work in consequence of having been recently poisoned (Nos. 1 and 11). It will be noticed that of the 13 men examined none had been employed for less than one year, the longest being 15 years. Of these thirteen men, 8 had never been off work at any time, but all had suffered at some time or other from symptoms of anilin poisoning; those most frequently complained of being headache, drowsiness, nausea, want of appetite, shortness of breath, palpitation, and tingling sensations in the feet and legs. In both the factories from which the cases were taken there was evidence that anilin vapour was present in the atmosphere. All the unpainted deal woodwork was stained a bright yellow colour which was said to be due to the anilin fumes in the air. In order to verify this statement strips of unpainted deal were exposed to anilin vapour in the laboratory when it was found that they quickly became stained yellow in the presence of even very small amounts of anilin vapour, thus giving a very delicate test for detecting its presence in the air. In every case the blood was examined spectroscopically but in no case was the spectrum of met-haemoglobin found to be present nor were any of the men cyanosed. Subsequent experiments proved however that the met-haemoglobin band could not be detected unless that substance was present in at least the proportion of 1 to 10 of oxy-haemoglobin.

The specific gravity of the blood was taken in every case and the percentage of haemoglobin calculated from it by means of Hammer-schlag's table, this being the only available method of estimating it and one which gives fairly accurate results; from this the colour index was determined.

The red corpuscles and leucocytes were counted with a Thoma-Zeiss haemacytometer, and the differential percentage count of the leucocytes was made from films stained by Jenner's method.

Among the anilin dyers the evidence of blood destruction was not very apparent from the blood counts. It will be seen from Chart No. I

TABLE I. *Anilin dyes.*

Case	Age	Length of employment	Off work	Cyanosis	Pallor	Specific gravity	Colour Index	Red corpuscles	Leucocytes Thousands	Eosinophils	Polymorpho- nuclears	Lymphocytes	Large mono- nuclears	Mast	Basophil reds
1	30	1	3	0	0	1056	1.0	4,448,000	7,280	.6	70	23	5	.3	+
2	34	1½	0	0	+	56	.8	5,100,000	5,160	.6	66	30	5	.3	-
3	68	2	1	0	+	58	.8	5,400,000	10,000	1.6	63	30	6.3	1.0	-
4	26	2½	1	0	+	52	.6	4,776,000	10,840	4.3	66	26	4.3	1.3	-
5	38	3	0	0	+	56	.8	4,840,000	9,960	4.3	70	22	3.3	.5	+
6	25	4	0	0	+	51	.8	4,456,000	12,440	5.3	43	48	3.6	2	+
7	46	4	0	0	0	54	.7	5,200,000	10,800	4	50	44	1.7	1	+
8	56	4	0	0	+	54	.7	4,464,000	4,240	0	50	47	2.5	2.5	-
9	27	4	0	0	+	48	.5	4,944,000	12,680	.6	80	17	3.6	.6	-
10	55	9	4	0	+	51	.6	5,280,000	6,640	1.6	69	22	5.3	1.3	-
11	28	9	1	0	0	56	.7	5,680,000	11,080	2.3	60	34	2.7	.3	-
12	46	12	2	0	+	51	.8	4,400,000	6,800	1.3	54	37	2.6	.9	+
13	35	15	1	0	0	58	.8	5,222,000	9,440	3.6	52	33	5.3	.3	+

that 6 out of the 13 cases examined had more than the normal number of red corpuscles per c.mm., the highest being 5,600,000 and the lowest 4,400,000. In all probability the effect of absorbing small doses of anilin daily, is to stimulate the production of red corpuscles by the bone-marrow, so that destruction is counter-balanced by renovation. Evidence of this was supplied by the low colour index and imperfect development of the corpuscles. None of the men showed signs of cyanosis at the time of examination, but nine of them showed conjunctival pallor. The specific gravity of their blood showed that there was a decrease in haemoglobin of from 5% to 50%. The colour index in the cases examined was with one exception below unity.

The number of leucocytes per c.mm. did not show any great departure from the normal; eight were above the average and five below. The highest was 12,000 and the lowest 4,000 per c.mm.

Examination of the stained blood films gave the most important indications of degeneration in the red corpuscles.

In most of the cases the variations in size of the corpuscles were considerable, ranging from  $5\ \mu$  to  $11\ \mu$ , the larger sizes being the most numerous. None showed any poikilocytosis but 6 out of the 13 showed basophil granulations in the red corpuscles. The number of cells affected varied from two or three in the whole film in the slight cases to 10 or 12 in every field of the microscope in the more pronounced ones. This reaction is of great significance, as it appears to be in anilin poisoning, as it is in lead poisoning, the earliest noticeable sign in the blood. No nucleated red corpuscles were found in any case, nor any pathological leucocytes.

The differential percentage leucocyte count showed a departure from the normal in several cases.

The number of polymorphonuclear cells showed a decrease in 9 out of the 13 cases; the decrease being considerable in cases 6, 7, 8, 12 and 13. There was a corresponding increase in the lymphocytes in these cases.

The mast cells were present in higher numbers than is normal in 6 of the 13 cases, but there does not appear to be any relation between these cases and those in which the polymorphonuclears were diminished. The eosinophils showed a percentage above the normal in three cases. Case No. 6, which showed the smallest percentage of polymorphonuclears, had the largest number of eosinophils, lymphocytes and mast cells.

III. *Conclusions concerning anilin.*

In anilin workers the following appear to be the most important points which are shown by a blood examination :

(1) An increase in the number of red corpuscles when the amount of poison absorbed is small and constant.

(2) A decrease in the specific gravity and haemoglobin of from 5 % to 50 %.

(3) A low colour index, showing that renovation of the corpuscles is proceeding more rapidly than the manufacture of haemoglobin.

(4) Degeneration or imperfect development of the red corpuscles as shown by the variations in size and the presence of corpuscles containing basophil granulations.

(5) Abnormal leucocytic percentages, consisting principally in a diminution in the polymorphonuclears and an increase in the other cells, particularly the lymphocytes, eosinophils and mast cells.

IV. *Nitro-benzine Workers.*

The 21 men examined were employed in the manufacture of di-nitro-benzol, as this substance is generally believed to be the most toxic of all the nitro-benzine series.

The men employed in the works visited are constantly shifted so that they do not work in the di-nitro-benzol department for more than a week or two at a time in consequence of the number of cases of poisoning which have occurred.

The examination was conducted on the same lines as that of the anilin dyers and the same facts noted. It will be noticed that the average length of employment is considerably shorter in the nitro-benzine workers than in the anilin dyers. Five of the 21 cases examined had been employed for one week or less, while only four had been employed for more than one year. This is no doubt owing to the dangerous nature of the trade. Very few workers escape poisoning at some time or other and so men do not care to remain long at it.

Excluding the five men who had been employed one week or less, 11 out of the remaining 16 had been off work at least once in consequence of being poisoned. All the men, including those who had not left their work, had suffered from some symptoms of poisoning, such as headache, drowsiness, loss of appetite, nausea, shortness of breath,

palpitation, cyanosis and pains in the legs. Of the cases recorded in Table II, No. 1 had only been employed for one day and therefore serves as a control case for purposes of comparison with the others, his blood being quite normal. Of the other 20 cases, 13 showed some degree of cyanosis; in most of them it was slight and confined to the lips, but in cases 6 and 8 it was well-marked. In these two cases spectroscopic examination showed the band of met-haemoglobin: in none of the others was it visible.

Most of the men had conjunctival pallor, the exceptions being those employed for one week or less (Nos. 1, 2, 3, 4, and 5). The specific gravity of the men's blood was below normal except in the five previously mentioned cases.

The colour index was in most cases nearly normal, this being probably due to the fact that blood destruction was unaccompanied by regeneration.

The number of red corpuscles per c.mm. was normal in only five cases, these again being the men who had only been employed for a very short time. Most cases had from 4 to  $4\frac{1}{2}$  million per c.mm., whilst three were below four million, the lowest being 3,600,000.

The leucocyte count varied from 4,000 to 21,000 per c.mm., eight being above 10,000, five below 7,000, the rest between these figures.

The differential percentage leucocyte count showed the same features as in the anilin dyes.

Thirteen of the 21 cases showed a decrease in the number of polymorphonuclears and a corresponding increase in the lymphocytes. It will be observed that in cases 2, 5 and 6 (men who had only been employed for a very short time) there was already a considerable decrease in the polymorphonuclears. In four cases (Nos. 7, 8, 13 and 21) there was a marked increase in the number of eosinophils, but these cases do not seem to bear any relation to those in which there was a decrease in polymorphonuclears.

In ten cases (Nos. 2, 4, 6, 7, 8, 10, 13, 14, 17 and 21) there was a considerable increase in the number of mast cells. No myelocytes were seen in any case. Only in three were any nucleated red cells found and these in very scanty numbers.

The red corpuscles varied very much in size in nearly all cases (from  $3\ \mu$  to  $12\ \mu$ ), the larger sizes being the most common. In cases 4 and 15 some poikilocytes were seen. In 16 of the 21 cases basophil granulations were present in the red corpuscles, three exceptions being men who had been employed for less than a week. In some cases they



TABLE II. *Di-nitro-benzol workers.*

Case	Age	Length of employment	Off work	Cyanosis	Pallor	Specific gravity	Colour index	Red corpuscles	Leucocytes	Eosinophils	Polymorpho- nuclears	Lymphocytes	Large mono- nuclears	Mast	Basophil reds
1	30	Days	0	0	0	1060	1.0	5,120,000	8,640	2.3	71	26	1.3	3	-
2	42	4	0	0	0	58	1.0	4,840,000	7,200	1.6	55	37	4	1.3	-
3	36	4	0	0	0	60	1.0	4,976,000	5,240	1.6	71	25	2	3	-
4	27	7	0	0	+	60	1.0	5,120,000	8,840	2.3	66	26	3.6	1.0	+
5	22	7	0	0	0	58	.9	5,168,000	10,160	1	58	36	5	0	+
6	30	21	0	+	+	52	1.0	3,712,000	15,640	2.3	56	42	2.6	1	+
		Months													
7	45	1	0	+	+	54	.9	3,792,000	14,640	8.3	63	24	5	1.6	+
8	23	1½	1	0	+	52	1.0	3,864,000	14,480	10	63	26	1	1.6	+
9	30	2	1	+	+	55	.9	4,264,000	21,160	.6	60	37	1	0	+
10	24	3	0	+	+	52	.8	4,032,000	14,360	2.6	74	22	2.6	1.3	-
11	32	3½	1	+	+	55	.9	4,336,000	7,960	2.3	63	31	2.6	0	+
12	26	4	1	0	+	57	1.0	4,280,000	16,420	0	68	28	4	0	+
13	51	4	1	+	+	55	.8	4,784,000	12,320	9	54	31	3	1.3	+
14	53	5	0	0	+	55	.9	4,040,000	6,520	.3	55	40	4.6	2	+
15	29	9	1	+	+	56	.8	4,240,000	10,280	1	42	56	3	3	+
16	49	10	1	+	0	54	.8	4,880,000	9,360	2.3	63	31	2.6	0	+
		Years													
17	34	1½	1	+	+	57	1.0	4,448,000	9,320	.3	66	31	1	1.3	+
18	47	1½	1	+	+	54	.9	4,168,000	4,400	2.3	47	47	3	0	-
19	41	2	0	0	+	54	.9	4,256,000	6,240	1.2	60	30	4	0	+
20	35	8	2	0	+	57	.9	4,576,000	8,760	.5	72	25	2.5	0	+
21	42	10	1	+	+	52	1.0	3,880,000	5,960	7	50	40	2.6	1.5	+

were few in number, while in others, noticeably in Nos. 6 and 8, the affected cells were very numerous, as many as 20 being present in every field of the microscope. It will be noticed that in cases 4 and 5 men who had been working for only one week, and whose red corpuscles were normal in number, yet showed a distinct basophil reaction, emphasising the fact that this is the earliest detectable blood sign in di-nitro-benzol as in anilin poisoning.

Spectroscopic examination showed only the oxy-haemoglobin bands in 19 cases, while in two (Nos. 6 and 8) a faint band in the red indicated the presence of met-haemoglobin in a proportion of at least 1 to 10 of the oxy-haemoglobin.

#### V. *Conclusions concerning di-nitro-benzol workers.*

(1) Di-nitro-benzol is more toxic than anilin, and causes more cases of acute poisoning than any other of the nitro-benzine series.

(2) It quickly affects the men who work in it, even within one week.

(3) The occurrence of red corpuscles showing basophil granulations is the first recognisable sign in the blood of poisoning by this substance.

(4) The number of red corpuscles is reduced after a short time by 1 to  $1\frac{1}{2}$  million per c.mm.

(5) The specific gravity and haemoglobin content are reduced in about the same ratio as the red corpuscles; therefore the colour index is not far removed from unity.

(6) Some leucocytosis, principally lymphocytosis, occurs at some stage in chronic poisoning.

(7) Even when there is considerable cyanosis the presence of met-haemoglobin cannot be detected spectroscopically except in the more severe cases.

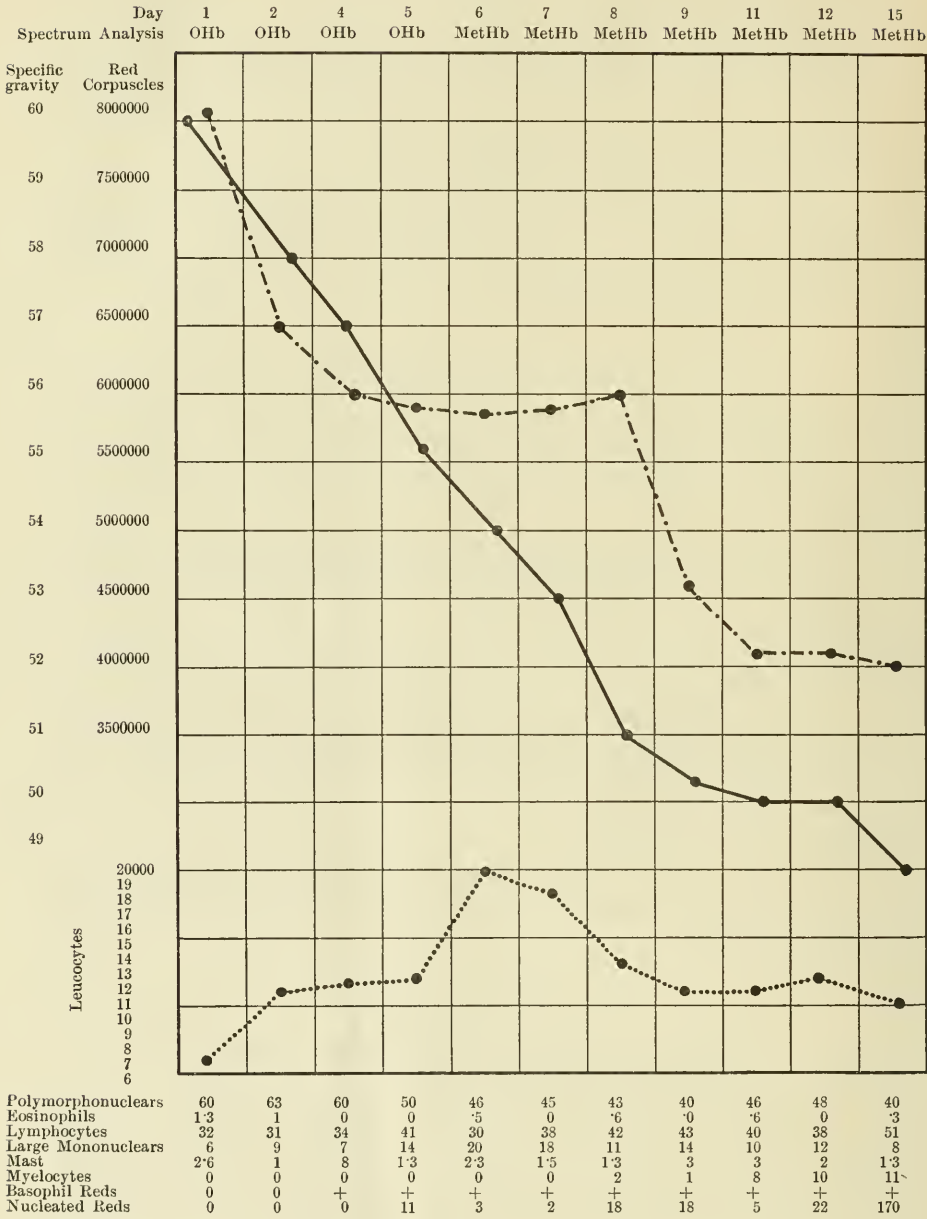
#### VI. *Animal experiments.*

In order to determine if possible the sequence of events in chronic anilin poisoning the following experiment was performed:

A rabbit weighing 2650 grammes was given subcutaneous injections of a 30% solution of anilin hydrochloride, and received 8 injections in 12 days; the initial dose was 0.1 c.c.: this was increased to .2 c.c. on the 5th day, and to .3 c.c. on the 6th and following days. On the last two days of the experiment the animal was placed in a chamber containing anilin vapour for half an hour.

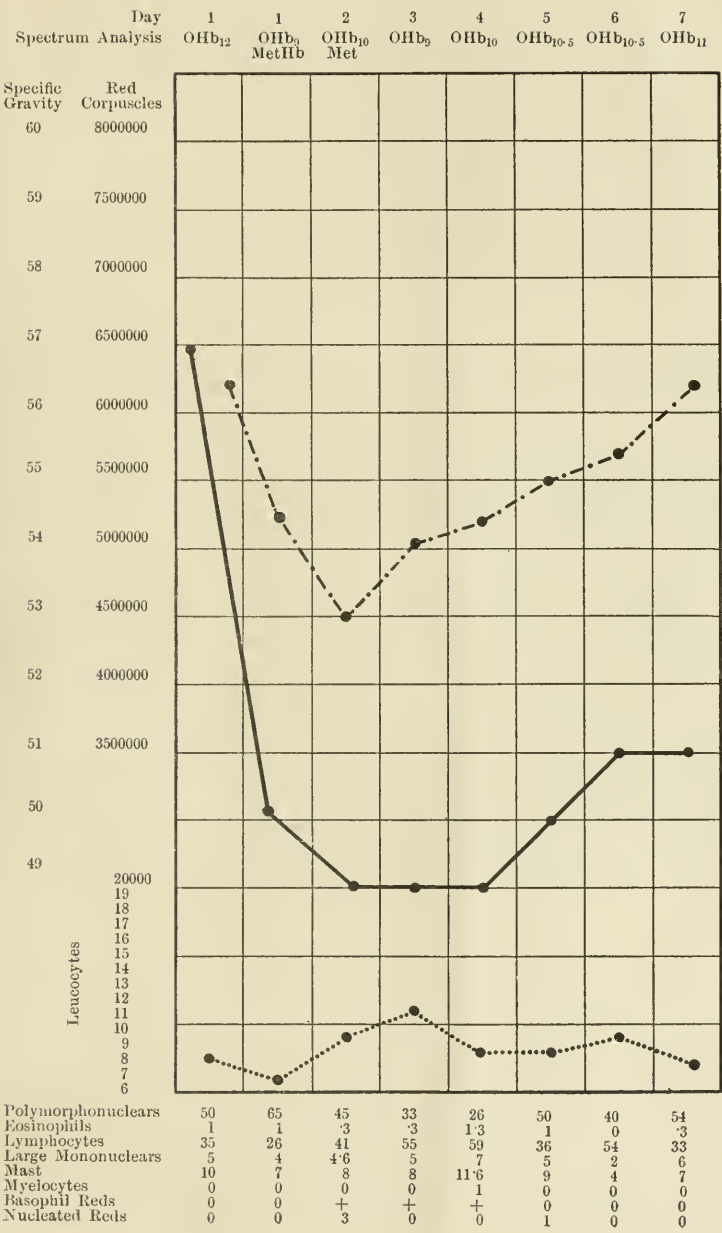
680 *Anilin Dyeing and Nitro-benzine Manufacture*

CHART I.



Thick line=specific gravity.  
Interrupted line=number of red corpuscles.

CHART II.



## 682 *Anilin Dyeing and Nitro-benzine Manufacture*

Chart I gives the results of this experiment.

There was a constant and progressive fall in the specific gravity of the blood (thick line).

For the first ten days the oxy-haemoglobin bands were the only ones visible spectroscopically. On the 11th day the met-haemoglobin band was seen and remained visible until the animal was killed.

The red corpuscles showed an almost continuous decrease in number, rapid at first, but slower later on (interrupted line).

The number of leucocytes increased for the first 6 days from 7,000 to 19,000 per c.mm. and after that gradually fell.

The differential percentage leucocyte count showed a progressive decrease in the number of polymorphonuclears, with a corresponding increase in the number of lymphocytes.

The large mononuclears increased in number up to the 6th day and after that decreased.

On the 8th day of the experiment polymorphonuclear myelocytes were found to be present in the blood and continued in increasing numbers until the animal was killed.

At no time were many basophil red corpuscles found, but on the fourth day polychromasia was present and continued to increase, until on the last day of the experiment there were as many cells showing this change as there were normal corpuscles. Poikilocytes were also present during the last six days of the animal's life.

On the sixth day nucleated red cells were seen and continued in increasing numbers until the last day of the experiment, when they were present in very large numbers.

In order to determine the time taken to recover from severe anilin poisoning the following experiment was performed :

A rabbit weighing 2850 grammes was placed in a chamber of anilin vapour at a temperature of 30° C. for one hour. Before the experiment began the animal's respirations were 72 and its heart beats 128 per minute.

On removal from the anilin chamber the animal seemed much exhausted and was unable to stand up ; its respirations were 158 per minute and of the Cheyne Stokes type, the heart beats were too rapid to count accurately, but were over 200 per minute.

The veins in the ears were much dilated and looked blue, as did also the nose and lips.

The blood examined spectroscopically gave the met-haemoglobin band.

One hour after removal from the anilin chamber the animal had nearly recovered and was able to move about, and on the following day it appeared quite well and took its food well. The met-haemoglobin band was still visible spectroscopically.

On the third day the met-haemoglobin band had disappeared.

The next experiment was undertaken in order to determine the sequence of events which occur in the blood after a single exposure to anilin vapour. The results are indicated in Chart II.



A rabbit was placed in a chamber containing anilin vapour at a temperature of 32° C. for 1½ hours. Examination of the animal's blood before the experiment began, showed it to be normal in every respect. After removal from the anilin chamber the animal was much exhausted and was unable to stand or sit but lay in any position in which it was placed. Its respirations were very hurried and of the Cheyne Stokes type. The veins in its ears were much dilated and its nose and lips were blue. The animal remained in a semi-comatose state for about two hours and after that time gradually recovered. Its blood was examined two hours after removal from the anilin chamber; the results of this examination are shown in the second column of Chart II. The fall in specific gravity was very marked; that in the red corpuscles less so and in the leucocytes still less. The met-haemoglobin band was well marked when the blood was examined spectroscopically.

The differential leucocyte count showed a slight increase in the percentage of polymorphonuclears, but was otherwise not much changed.

On the following day the animal had quite recovered and took its food well.

It will be seen from the chart that the specific gravity had continued to fall. The number of red corpuscles per c.mm. had fallen considerably but the leucocytes had somewhat increased. The differential percentage leucocyte count showed a considerable increase in the lymphocytes and a corresponding decrease in the polymorphonuclears. The red corpuscles showed great variations in size with a moderate number of polychromatic and basophil cells. Three nucleated red cells were seen. The met-haemoglobin band had disappeared from the spectrum. On the succeeding days the specific gravity remained at 1049 for two days and then gradually rose.

The red corpuscles quickly increased in numbers and on the seventh day were as numerous as they were before the experiment. The leucocytes increased in number till the third day and then diminished.

The polymorphonuclears decreased till the fourth day and after that appeared in normal numbers. The lymphocytes on the other hand increased till the fourth day and then diminished. The other leucocytes did not vary in any marked degree. Basophil reds were present for eight days and after that ceased to appear. One myelocyte was seen on the fourth day and one nucleated red cell on the fifth day.

### *Conclusions from animal experiments.*

These experiments show that anilin whether injected subcutaneously or inhaled as vapour very rapidly produces its destructive action on the blood.

This action is manifested by the following points in chronological sequence.

- (1) Production of met-haemoglobin in the blood.
- (2) Haemolysis and destruction of red corpuscles.
- (3) Rapid fall in the specific gravity and haemoglobin content of the blood, accompanied by a slight reduction in the number of leucocytes.

## 684 *Anilin Dyeing and Nitro-benzine Manufacture*

(4) A continued increase in the number of polymorphonuclears with a corresponding increase in the lymphocytes.

(5) The occurrence in the red corpuscles of basophil granules and polychromasia.

(6) The occurrence of nucleated red cells in severe cases.

(7) Recovery after severe poisoning is rapid and continuous and apparently no permanent disability is entailed.

### *Summary.*

These animal experiments corroborate the findings in the blood of anilin and nitro-benzine workers and enable us to follow the sequence of events, which appear to be the same in cases of poisoning by either of these bodies, though they are much more marked in the case of the latter.

These bodies may gain access to the body either by (1) inhalation of their vapours; (2) absorption through the skin; (3) absorption from the alimentary canal after being swallowed.

Probably the first method occurs most commonly, since cases of poisoning are much more frequent in hot close weather than during the colder seasons of the year. No matter how they are absorbed these bodies appear to have the same action on the blood, which is as follows:

They first convert the oxy-haemoglobin of the red corpuscles into met-haemoglobin, this is followed by haemolysis, degeneration of cytoplasm and escape of haemoglobin into the plasma.

If a considerable amount of met-haemoglobin is present in the blood, the respiratory capacity of the red corpuscles is diminished, the patient becomes cyanosed and the colour of the blood is changed to a chocolate brown. This fact renders the estimation of haemoglobin by the ordinary colour standards impossible, as they are based on the colour of pure oxy-haemoglobin, which is quite different from that of met-haemoglobin.

In the less severe cases of poisoning in which met-haemoglobin is not present in sufficient amount to be detected spectroscopically, the changes which may be found in the blood are sufficiently characteristic to enable a diagnosis of anilin or nitro-benzine poisoning to be made.

These consist in:

(1) A decrease in the percentage of haemoglobin as estimated from the specific gravity of the blood of from 5% to 50%.

(2) A decrease in the number of red corpuscles if the amount of poison absorbed is considerable ; if the dose is very small this decrease is not found, blood formation apparently keeping pace with blood destruction.

(3) Degeneration and imperfect development of the red corpuscles as shown by the occurrence of basophil granulations, polychromasia, poikilocytosis, and variations in size.

(4) The presence of nucleated red corpuscles in severe cases.

(5) A decrease in leucocytes rapidly followed by an increase ; the increase being principally due to the number of lymphocytes.

The simultaneous occurrence of all or several of these signs in the blood enables a diagnosis of poisoning by anilin or nitro-benzine to be made in quite mild cases.

Cessation from work for a short time enables the blood to recover rapidly from the effects of poisoning.

The best treatment in any severe case of poisoning by these bodies appears to be inhalation of oxygen.

Haldane, Makgill, and Mavrogordato<sup>1</sup> found that mice could be kept alive in oxygen at a pressure of two atmospheres even when 90 % of their blood had been converted into met-haemoglobin by injection of sodium nitrite. They also showed that animals showing severe symptoms of di-nitro-benzol poisoning recovered rapidly when placed in oxygen, and that as other substances besides met-haemoglobin are produced by the decomposition of the haemoglobin, the absence of a met-haemoglobin band is no evidence that the symptoms are not due to oxygen asphyxia.

Permission to publish this paper has kindly been given by Mr H. L. Samuel, Chairman of the Home Office Committee on Industrial Diseases.

<sup>1</sup> *Journ. of Physiology*, Vol. xxi. p. 160, 1897.

## PUBLICATIONS RECEIVED.

## BOOKS.

*Deutsches Bäderbuch* bearbeitet unter Mitwirkung des Kaiserlichen Gesundheitsamtes von F. Himstedt ; E. Hintz ; L. Grünhut ; C. Jacoby ; H. Kauffmann ; K. Keilhack ; H. Kionka ; F. Kraus ; V. Kremser ; P. Nicolas ; Th. Paul ; F. Röchling ; A. Scherrer ; C. Schütze ; A. Winckler ; E. Rost ; G. Sonntag ; F. Auerbach und unter Beihilfe von K. von Buchka ; E. Dietrich ; O. Lassar ; E. von Leyden ; E. A. Merck. Mit 13 Tafeln graphischer Darstellung von Quellenanalysen, einer Übersichtskarte und der Hellmannschen Regenkarte. Verlag von J. J. Weber, Leipzig, 1907. 535 pp. 30 × 23 cm. Cloth.

This exhaustive work gives the fullest information about German Mineral Springs, Seaside and Health Resorts. Sections are devoted to the geology of the country and to the chemical, pharmacological, clinical, climatological and industrial problems involved. The Mineral Springs are all classified and their analyses given. The book is excellently indexed and provided with graphic charts of the chemical analyses, maps, etc., etc. The work is a monument of industry and destined to possess permanent scientific value. It will constitute the main work of reference for years to come.

DÖNITZ, W. (1907). *Die wirtschaftlich wichtigen Zecken mit besonderer Berücksichtigung Afrikas*. 127 pp., 38 figs., 6 plates. Leipzig : J. A. Barth. Price : unbound 5 M., bound 5.80 M.

A short but accurate and useful work upon the species of ticks concerned in the transmission of disease. The book contains much original matter.

FRIEDRICH, E. (1906). *Die Seereisen zu Heil- und Erholungszwecken, ihre Geschichte und Literatur*. Berlin : Vogel and Kreienbrink. 325 pp.

This work presents a detailed study of the history and literature of the subject of the effects of sea-climate in health and disease. A mass of facts are brought together from scattered sources in a manner that will save much labour to those who follow in the author's footsteps.

GRIMSHAW, J. (v. 1907). *Eye strain and Eyesight*. How to help the Eye and save the Sight. London : J. & A. Churchill. 71 pp., numerous figs. 1/- net. Cloth bound 2/-.

A pamphlet "written for the general public ; but especially for the parent, teacher, and publicist."

- LAVERAN, A. (1907). *Traité du Paludisme*, 2<sup>d</sup> ed. 622 pp., 58 text figs. and 1 coloured plate. Paris: Masson et Cie.

The second edition of this well-known work shows signs of considerable revision and much new matter has been added so as to bring the book up to date. The work will be welcome to those who are concerned in the study of malaria.

- MPVAIL, J. C. (1907). *The Prevention of Infectious Diseases*. London: Macmillan & Co., Ltd. 290 pp., numerous figs., maps and diagrams. 22 × 14 cm. Price 8/6 net. Cloth.

This book contains the Lane Lectures delivered at the Cooper Medical College San Francisco, in 1906, and revised for publication. The author begins his preface with the modest words "This is not a treatise on Infectious Diseases, but only a course of lectures about their prevention and control." The ten lectures deal with Public Health Organisations in Britain and with the prevention of Typhus Fever, Enteric Fever, Plague, Measles, Scarlet Fever, Diphtheria, Small-pox, and Tuberculosis. The lecture style renders the book easy reading and even a cursory glance through its well-printed pages will convince the reader that much interesting information is to be obtained from a perusal of the work.

- MANSON, Sir PATRICK (1907). *Tropical Diseases. A Manual of the Diseases of Warm Climates*. London: Cassell & Co., Ltd. 876 pp., with 7 coloured plates and 241 plain figs. 19 × 13 cm. Price 12/6 net. Cloth.

The fourth edition of this well-known work will be welcomed by students of tropical medicine. A great deal of new material has been added and the book has undergone thorough revision.

- MERRIMAN, M. (1906). *Elements of Sanitary Engineering*. (3rd ed., enlarged. First thousand.) New York: John Wiley & Sons. London: Chapman & Hall, Ltd. 250 pp., numerous illustrations. 23 × 15 cm. Price 9/- net. Cloth.

This work is primarily intended for students in engineering colleges but it may well appeal to a wider class of readers. At the end of each chapter are given exercises and problems for students. The book is divided into six chapters dealing with Sanitary Science—Water—Water-supply Systems—Sewage Systems and Disposal—Refuse and Garbage. The work should prove useful to those desiring a condensed account of the elements of sanitary engineering.

- MUIR, R. and RITCHIE, J. (1907). *Manual of Bacteriology* (4th edition). Edinburgh & London: Young J. Pentland. 605 pp., 171 illustrations. 19 × 13 cm. Cloth.

The new edition of "Muir and Ritchie" has been considerably revised and additions have been made so as to keep the book abreast of the times. This excellent Manual is to be highly recommended to students of medicine.

- PARTRIDGE, W. (1907). *The Bacteriological Examination of Disinfectants*. London: The Sanitary Publishing Co., Ltd. 66 pp., 4 figs. 19 × 13 cm. Price 2/6 net. Cloth.

This little work deals more especially with a description of the Rideal-Walker method of testing disinfectants, and is intended to be of use to medical officers of health and to sanitary inspectors as well as to laboratory workers. A short preface is contributed by Major C. E. P. Fowler, R.A.M.C.



- PROWAZEK, S. v. (1907). *Taschenbuch der mikroskopischen Technik der Protisten-untersuchung*. Leipzig: Verlag von Johann Ambrosius Barth. 66 pp. 17×11 cm. Price 2 M. Cloth.

This little book contains much information in condensed form and it will be very useful, especially to those engaged in the study of the Protozoa.

- STEPHENSON, S. (1907). *Ophthalmia Neonatorum*, with especial reference to its causation and prevention. London: George Pulman & Sons, Ltd. 258 pp. 24×15 cm. Cloth.

This book represents the Middlemore Prize Essay of the British Medical Association, 1907. The literature of the subject is very fully gone into and as a digest alone it will be of value to those desiring a more detailed knowledge of the diseases which are grouped under the name of ophthalmia neonatorum.

- WHIPPLE, GEORGE C. (1907). *The Value of Pure Water*. New York: John Wiley & Sons. London: Chapman & Hall, Ltd. 84 pp., 19 tables. 20×14 cm. Price 4/6 net. Cloth.

This little book consists of a compilation of scientific papers published elsewhere by the author. The writer has adopted the "financial standard" in judging of the quality of water supplies, and consequently presents many interesting facts which are not included in works on water supply. A knowledge of these facts may well be of use to the practical sanitarian.

## NEW JOURNALS.

- The All India Hospital Assistants' Journal* (1907). The Journal of the All-India Hospital Assistants' Association. (Monthly.) Editors: P. S. Ramchandrier, and Anant Santram Malve. Vol. 1. Nos. 1-4, 119 pp. Bombay: The Patriot Machine Printing Works.

- The Calcutta Medical Journal* (III. 1907). (The Journal of the Calcutta Medical Club.) Vol. 1. No. ix., 257-288 pp. Price 8 Annas. Calcutta: The Calcutta Medical Club. 72, Harrison Road. (Published monthly.)

- Il Ramazzini Giornale Italiano di Medicina Sociale*. Edited by C. Biondi, L. Borri, G. Gasperini, G. Y. Giglioli and G. Pieraccini. Published monthly by L. Niccolai, Florence. Subscription 15 Lira abroad, 2 Lira for single numbers. Vol. 1. No. 1., Jan. 1907, pp. 1-104.

The first number of this Journal contains the following papers: Lo studio antropologico delle classi povere, by A. Niceforo; Antagonismo igienico-economici, by A. Celli; Dalle "malattie degli artefici" alla "patologia del lavoro," by G. Y. Giglioli; Il concetto medico giuridico del "rischio professionale" rispetto alla legge sugli infortuni del lavoro, by L. Borri; Il problema medico-legale della responsabilità per le malattie del lavoro, by G. Sanarelli; Della somministrazione gratuita dei medicinali ai poveri e della municipalizzazione del servizio farmaceutico, by G. Pieraccini; Di una particolare tendosinovite dei lavoratori dei campi, by F. Pellizzari; L'igiene industriale e la protezione contro le malattie del lavoro nella Svizzera.

*La Revue Médico-Sociale.* A bimonthly journal edited by Prof. O. Laurent and J. Croco, and published by Hayez, Imprimeur des Académies royales, rue de Louvain, Brussels. Subscription abroad 7 francs a year. 35 x 27 cm. Vol. I. No. 1 appeared 10 Jan. 1907.

The Revue contains original papers and reviews of current literature and progress in medicine, surgery, hygiene, etc.

## CURRENT JOURNALS, ETC.

*Atti della Società per gli Studi della Malaria.* Vol. VIII, containing 8 coloured plates and many text figures. Rome: Società per gli Studi della Malaria. 896 pp.

Contains 47 original papers and reports dealing with Malaria and its prevention in different parts of the world.

*Archives de l'Institut Pasteur de Tunis* (iv. '07). Part II. pp. 55-122, 1 plate, 10 figs., 1 map. Tunis: Imprimerie Moderne (J. Orliac).

Contains: La lèpre en Tunisie by Drs Bastide and C. Nicolle; Appendix thereto by Dr Conseil.

*Boletín del Instituto de Alfonso XIII.* (31. III. 1907). (Sueroterapia, Vacunación, Bacteriología.) Año III. No. 9. Edited by D. Santiago Ramón y Cajal. 63 pp., 3 coloured figs. Madrid: A Marzo.

Contains: La luza ó geluza (una enfermedad de las cabras) by F. Murillo and D. García é Izcara.—Reviews.—Statistics relating to rabies cases treated at the Institute.

*La Bulgarie Médicale.* Revue mensuelle. Année I. Nos. 11, 12. (November, December 1906.) Edited by Dr Chr. Doctoroff. pp. 161-192, fig. 21. Sofia: Imprimerie de la Cour.

Contains amongst other papers: Quelques données statistique sur l'état sanitaire de la ville de Sofia pendant 1904-1907, by Dr Michaloff; Sur une épidémie de tuberculose by Dr Vasseff.

## REPORTS.

*Bristol Port Sanitary District. Annual Report* (1907) of the Medical Officers of Health, and of the Chief Port Inspector of Nuisances, for the year 1906, including Report on Canal Boat Inspection. Bristol: Bennett Brothers, Ltd., Printers. 33 pp., 4 tables.

*6th Annual Report* (1906) of the New York State Hospital for the care of Crippled and Deformed Children for the year ending September 30, 1906. Albany: J. B. Lyon Co. 29 pp., 7 plates.

*10th Annual Report of the Loomis Sanatorium and Annex.* (For the Treatment of Tuberculosis.) (31. x. 1906.) Liberty, Sullivan County, New York. 49 pp., 14 plates.

*Medical Supplement to the Tenth Annual Report of the Loomis Sanatorium and Annex* (31. x. 1906). (Prepared in accordance with the suggestion of the National

- Association for the Study and Prevention of Tuberculosis. Liberty, Sullivan County, New York. 11 pp.
- BANNERMAN, W. B. (1907). *Report of the Bombay Bacteriological Laboratory* (formerly Plague Research Laboratory) for the Nine Months ending 31st December, 1906. Bombay: Printed at the Government Central Press. 22 pp. Price 6d.
- CHALMERS, A. J. (1907). *Report on the Sanitation of Colombo and the Causes of Abnormal Incidence of Specific Diseases in 1906*. Colombo: Printed by H. C. Cottle. 68 pp., 8 maps, 5 plans, 24 photographs. Price 2.50 Rs.
- CHAPIN, C. V. (1906). *Sanitary Legislation in the United States enacted during the year 1906*. (Special Bulletin of the State Board of Health. State of Rhode Island and Providence Plantations.) Providence: E. L. Freeman Co., Printers to the State. 102 pp. 23 × 15 cm. Cloth.
- CHAPIN, C. V. (1906). *Fifty-first Annual Report upon the Births, Marriages, and Deaths in the City of Providence for the year 1905*, including Tables for fifty years. (City Document, No. 23.) The Providence Press: Snow & Farnham, City Printers. 239 pp., 24 tables. 22 × 15 cm.
- DAVIES, D. S. (1907). *City and County of Bristol. Annual Report of the Medical Officer of Health, and of the General Medical Superintendent of the City Hospitals*. Bristol: Bennett Brothers, Ltd., Printers. 146 pp. Numerous tables. 24 × 16 cm. Cloth.
- ELKINGTON, J. S. C. (1906). *Report on the results of a medical examination of over 1200 children attending State Schools in Hobart*; with notes on a similar examination of 51 children attending the Campbell Town State School, and of the 35 inmates of the Boys' Training School. Tasmania: John Vail, Government Printer, Hobart. No. 23. 26 pp.
- ELKINGTON, J. S. C. (1906). *Outbreak of Milk-borne Typhoid at Zeehan, 1906*. Tasmania: John Vail, Government Printer, Hobart. No. 48. 9 pp.
- FREMANTLE, F. E. (VIII. 1907). *Annual Report of the Medical Officer of Health for Hertfordshire for the year 1906*. Hertford: Stephen Austen & Sons, Ltd. 177 pp., 42 tables. 24 × 15 cm.
- Imperial Cancer Research Fund. Fifth Annual Report for the year 1906-7* (I. VII. 07). Printed by Taylor & Francis, Red Lion Court, Fleet Street, London. 18 pp.
- JUILLERAT, P. (II. III. 1907). *Préfecture du Dép't de la Seine. Rapport à M. le Préfet sur les Recherches effectuées au Bureau du Casier sanitaire pendant l'année 1906 relatives à la répartition de la tuberculose dans les maisons de Paris*. Paris: Imprimerie et Librairie Centrales des Chemins de fer. Imprimerie Chaix. 19 pp.
- JUILLERAT, P. and BONNIER, L. (II. III. 1907). *Préfecture du Dép't de la Seine. Rapport à M. le Préfet sur les Enquêtes effectuées en 1906 dans les maisons signalées comme foyers de tuberculose*. Paris: Imprimerie et Librairie Centrales des Chemins de fer. Imprimerie Chaix. 6 pp.
- MURISON, P. (1906). *Durban Corporation. Medical Officer's Report for the Municipal Year ending 31st July, 1906*. 27 pp., 5 charts. Durban: P. Davis & Sons.
- NEWSHOLME, A. (1907). *Annual Report on the Health, Sanitary Condition, &c. of the County Borough of Brighton, for the year 1906*. Brighton: Brighton Society & Guardian Press, Ltd., 34, North Street. 90 pp., 2 figs., 1 chart.

PORTER, C. (1906). *Report of the Medical Officer of Health on the Public Health and Sanitary Circumstances of Johannesburg during the Period 1st July, 1904—30th June, 1906.* To which is appended "A Report by the Medical Attendant (Dr P. G. Stock) on the Health of the Natives and Indians employed by the Council." 71 pp., with charts. Johannesburg: E. H. Adlington & Co.

*Proceedings* (1907) *of the Conference on the Teaching of Hygiene and Temperance in the Universities and Schools of the British Empire*, held at the Examination Hall, Victoria Embankment, S.W. London: John Bale, Sons & Danielsson, Ltd. 129 pp. 19 x 12 cm. Price 2/- net. Cloth.

The Conference whose proceedings are the subject of this little book was brought about primarily by those interested in securing the teaching of Hygiene and Temperance in the Elementary and Secondary Schools of the United Kingdom. The meeting was held in April, 1907, at the time of the Colonial Conference, with the result that help was obtained from the officially appointed delegates from the Colonies and from others interested in this branch of education in different parts of the Empire and in European countries. It seems curious that the Schools and not the Universities of this Country should take the lead in enlightening the people. This Report of the Proceedings of the Conference will be of value for further reference to all interested in the subject.

ROSENAU, M. J., LUMSDEN, L. L. and KASTLE, J. H. (1907). *Report on the Origin and Prevalence of Typhoid Fever in the District of Columbia.* (Treasury Dept Publ. Health and Mar.-Hosp. Service U.S., Hygiene Laboratory.—Bulletin No. 35.) Washington: Government Printing Office. 361 pp. Numerous maps, charts and diagrams.

*Scientific Reports by Members of the Medical Staff.* (Sanitary, Maritime and Quarantine Council of Egypt.) Edited by M. A. Ruffer (President of the Council). (1906.) Alexandrie: Société de Publications Égyptiennes. 206 pp.

Contains the following original papers: On the Diagnosis of Vibrios, by M. Crendiropoulo.—Ueber Cholera- und choleraähnliche Vibrionen unter den aus Mekka zurückkehrenden Pilgern. Ein Beitrag zur Epidemiologie der Cholera, by F. Gotschlich.—Report on the Bacteriological Researches at El Tor during the Pilgrimage season of 1906, by F. Gotschlich.—Note on six Vibrios isolated from water of ships calling at Port-Said, by G. Zirolia.—A contribution to the study of the presence and formation of agglutinins in the blood, by M. A. Ruffer and M. Crendiropoulo.—On agglutination of Vibrios, by M. Crendiropoulo and Miss B. S. Amos.—Further observations on the influence of Calcium Chloride on the Agglutination of Vibrios, by M. Crendiropoulo and Miss B. S. Amos.—On Haemolytic and Haemosozic serums, by M. A. Ruffer.—On the Toxic properties of Bile and on Antihaemolytic (Haemosozic) serum, by M. A. Ruffer and M. Crendiropoulo.—Note on haemosozic sera, by M. A. Ruffer and M. Crendiropoulo.—On substances favouring and inhibiting the Action of the Haemolysins of Bile and Serum, by M. A. Ruffer and M. Crendiropoulo.—Sur le pouvoir hémosoïque du chlorure de sodium et son mode d'action, by M. A. Ruffer and M. Crendiropoulo.—On the lysogenic and haemosozic properties of urine, by M. A. Ruffer, M. Crendiropoulo and G. Calvo-coressi.—On a hitherto undescribed change in the urine of patients suffering from Nephritis,



by M. A. Ruffer and G. Calvocoressi.—Notes on the lesions produced by *Oxyuris vermicularis*, by M. A. Ruffer.—On the effect of ligature of one ureter, by Miss B. S. Amos.—Contribution to the technique of Bacteriology, by M. A. Ruffer and M. Crendiropoulo.—Note on the dialysis of toxins through collodion walls, by M. A. Ruffer and M. Crendiropoulo.—On some results obtained by disinfection and isolation against Cholera, by M. A. Ruffer and C. Zachariades Bey.

YOUNG, C. W. F. (1907). *County Council of Middlesex. Annual Report of the County Medical Officer of Health for the year 1906, including a Summary of the Annual Reports of the District Medical Officers of Health.* London: Harrison & Sons. 265 pp., 10 diagrams, 3 Tables, 1 map. 21 × 13 cm. Boards.

### REPRINTS, ETC.

BENTMANN and GÜNTHER (1907). Beiträge zur Kenntnis des *Trypanosoma gambiense*. *Archiv f. Schiffs- u. Tropen-Hygiene.* Bd. xi. 70 pp., 2 plates.

FOX, G. H. (iv. 1907). Diet as a Therapeutic Measure in Diseases of the Skin. *Journ. of Cutan. Dis.* 6 pp.

HILL, E. and HAYDON, L. G. (III. 1907). A Contribution to the Study of the Characteristics of Larvae of Species of Anophelina in South Africa. *Annals of the Natal Government Museum*, Vol. I., Part 2. pp. 111–157, Plates XV–XXVI.

JOHANNSEN, O. A. (II. 1907). Tests of Water by Bacterial Inoculation. *Amer. Med. N. S.*, Vol. II. pp. 112–116.

KEETLEY, C. B. (1907). *The Prevention of Cancer and its Relation to that of some other Diseases and Calamities.* London: Balliere, Tindall & Cox. 38 pp. 21 × 13 cm. Price 1/- net. Boards.

VIERECK, H. (1907). Studien über die in den Tropen erworbene Dysenterie. *Archiv f. Schiffs- u. Tropen-Hygiene.* 41 pp., 3 plates.

WERNICKE and WELDERT (1907). Untersuchungen über das von Wernicke angegebene Verfahren der gegenseitigen Enteisung und Entbräunung von eisenhaltigen und durch Huminstoffe braun gefärbten Grundwässern. *Mitteil. a. d. Königl. Prüfungsanstalt f. Wasservers. u. Abwässerbeseitigung zu Berlin.* Heft 8. 28 pp.



REPORTS ON PLAGUE INVESTIGATIONS  
IN INDIA.

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA, THE  
ROYAL SOCIETY, AND THE LISTER INSTITUTE.

(Plates XIX—XLI, with seventy-six Maps and Charts.)

*(Continued from Volume VII, p. 476.)*

	PAGE
XXI. Digest of recent observations on the epidemiology of plague . . . . .	694
XXII. Epidemiological observations in Bombay City . .	724
XXIII. Epidemiological observations in the villages of Sion, Wadhala, Parel and Worli in Bombay Village .	799
XXIV. General considerations regarding the spread of infec- tion, infectivity of houses etc. in Bombay City and Island . . . . .	874
XXV. Epidemiological observations in the villages of Dhand and Kasel (Punjab) . . . . .	895

## XXI. DIGEST OF RECENT OBSERVATIONS ON THE EPIDEMIOLOGY OF PLAGUE.

- I. Introduction.
- II. The epizootic amongst rats.
  1. The general relationship between the epizootic and the epidemic.
  2. The mode of infection of rats in nature.
  3. The course taken by the epizootic.
  4. The natural history of rats in relation to the epizootic.
- III. The mode of entrance of the plague bacillus into the human organism.
- IV. The mode of spread of the infection.
  1. Direct contact with the patient suffering from plague.
  2. Infectivity of houses.
  3. The spread of infection by indirect means, *e.g.* clothing, food, merchandise, etc.
  4. The importation of infection into a hitherto uninfected locality.
- V. Certain alleged contributory causes of the spread of the infection.
  1. Influence of insanitary conditions.
  2. Occurrence of plague in domestic and other animals.
- VI. The seasonal prevalence of plague.

### I. INTRODUCTORY.

The history of the study by modern methods of the epidemiology of plague may be said to date from the discovery of *B. pestis* by Yersin and Kitasato in 1894. The severe outbreaks which since then have yearly recurred, notably in Bombay and Hongkong, have offered a wide field for investigation. That the opportunities thus afforded have not been neglected is sufficiently shown in the statement that in Bombay alone no fewer than six scientific commissions devoted their attention to various aspects of the disease within a few years. In addition, epidemics have been investigated in many places throughout the world.

Perusal of the extensive literature dealing with the epidemiology of plague makes it evident that many conflicting opinions have been held within recent years by workers in this field: especially

is this the case with regard to the all-important question of the mode of infection in man and in rats. The reason for this confusion is not far to seek. In the first place, even at the present time there is no unanimity of opinion as to the exact rôle which ought to be attributed to the rat in epidemics of plague in man. In the second place those who champion the view that the rat is an important agent in the spread of plague are confronted with the difficulty of knowing precisely in what way the infection is conveyed from rats to man. Additional complexity has been imparted to the problem of the mode of transmission of infection from the rat to man by the suggestion, first made in 1897 and since then widely discussed, that the flea might act as an intermediary between the rat and man. In the third place, it has seemed to some that a disease which is frequently septicaemic must be contagious; the partisans of this view look upon the plague patient as the chief source of danger. Lastly, there are those who seek to explain such facts as the persistence of infection in a locality or the importation of infection into a locality from far distances, on the view that the plague bacillus is capable of living as a saprophyte in soil, in clothing, or in articles of merchandise.

It cannot be doubted, that it has often proved difficult to reconcile apparently well founded observations in the epidemiology of the disease with any one theory of infection.

We propose in the account that follows to make a survey of the conclusions arrived at by those who have worked at the subject during the last 10 or 12 years. Within the compass of this survey cognisance will be taken only of the views of these observers on what we believe to be essential points in the epidemiology of the bubonic and septicaemic varieties of the disease.

The subject matter has been arranged in sections, each having reference to a definite aspect of the problem. In each section the views held by different workers on the question under discussion are summarised; the countries in which any observations have been made being dealt with separately and in a regular order. It is hoped that in this way the reader will obtain a clear idea of the different conclusions which have been arrived at on any one point, as well as an impression of the opinions of the individual workers on the whole problem.

## II. THE EPIZOOTIC AMONGST RATS.

1. *The General Relationship between the Epizootic and the Epidemic.*

*India.* The importance of the epizootic in the spread of plague in Bombay was early recognised by Snow and Weir (1897), Hankin (1898) and the German Commission (1899). On the other hand the members of the Commission (1897) sent from Egypt to study the disease reported that the part played by rats was most probably a very small one, the plague patient being the chief source of danger. Simond (1898) from his observations of the disease in India assigned to the rat the essential rôle in the dissemination of plague. Hankin (1898) published his views a few months later than Simond. He pointed out that the incidence of plague bore definite relation to the accessibility of man for rats. In a later paper (1905) he appears to have modified his views, since he states his belief that rats are not a necessary cause or agent in the spread of plague. Hankin further adopts the view that there is no quantitative relation between rat and human mortality and he has cited several instances which seem to support this belief. Koch (1898) paid a brief visit to India in 1897 and as a result emphasised the "very important fact" that rats are essentially concerned in the spread of the disease (*ganz wesentlich betheiligt sind*). In a later contribution (1901) he defined plague as a disease of rats in which men participate.

The Indian Plague Commission (1901) summed up the evidence presented to them in words to this effect. The chief importance of rats in the epidemiology of plague seems to arise in connection with the first outbreak of the disease in an infected place. When plague is once established in a place human agency is a more important factor in spreading the disease than the agency of rats.

As plague became scattered over India opportunities were given for its study under very varying conditions and as a consequence many valuable observations bearing on the point under discussion were made by the various Sanitary Officers, notably by James (1899), and are to be found in the official reports to the Indian Government. In an excellent summary of these reports by Bannerman (1906), from which we shall frequently have occasion to quote, the statement is made that evidence derived from Indian experience incriminating the rat as an agent in the spread of plague is overwhelming. J. A. Turner (1905), from an intimate acquaintance as Medical Officer of Health with the conditions

in the native city of Bombay, stated his conviction that rats play an important part in the spread of plague. Browning-Smith (1906), who has had a large experience as a plague officer in India, has affirmed recently that in the Punjab bubonic plague in epidemic form is associated with and is dependent on an epizootic in rats.

*Australia.* The relationship between the epizootic and epidemic has been studied with particular care for the outbreaks which have occurred in Sydney since 1900 by Ashburton Thompson, aided on the experimental side of the work by Tidswell.

The value of the observations made by Thompson is enhanced by the continuous examination on an extensive scale of rats caught in the infected quarters of the city. On account of the comparatively small number of human cases in each epidemic a detailed investigation of each case, especially in its relation to plague-infected rats, was possible. For this reason and on account of the evident exactitude with which the work was carried out the conclusions derived by Thompson from his observations of plague in Sydney deserve careful consideration.

These conclusions may be stated thus:

- (1) Plague in rats preceded the first case which occurred in man.
- (2) The epizootic area was practically co-extensive with the epidemic area.
- (3) The epidemic is due to communication of infection from rat to man.

Thompson has rightly drawn attention to an important point which we shall illustrate later from our own experience, namely, the danger of concluding that plague rats are absent from an infected locality because none have been found. It is obvious that this depends altogether on the extent and the thoroughness of the search for dead rats and upon the care taken in the subsequent examination of any such which may be found. There can be no doubt that a failure to recognise the importance of this matter has more than once given rise to mistaken ideas upon the subject of the relationship between plague-infected rats and man.

Baxter-Tyrie (1905) investigated outbreaks of plague in Brisbane on lines similar to those carried out at Sydney. He holds the rat responsible for conveying infection to man.

*Hongkong.* A large amount of research has emanated from workers in Hongkong since the discovery of the specific bacillus in 1894.

Yersin (1897) remarks "Plague which is at first a disease of the rat soon becomes a disease of man."



Clark (1901) observed that dead rats could be found in plague houses before human cases occurred. He also noted that the general rat mortality went on increasing several weeks ahead of the human mortality.

Simpson (1903) concluded that infected rats play a part in the maintenance and dissemination of plague.

Atkinson, Pearce, and Hunter (1904) remarked the general correspondence between the epizootic and epidemic and stated that rat plague begins earlier and lasts longer than plague in man. Hunter (1904) made extensive observations on the occurrence of plague in rats in Hongkong during several years: in this way he was enabled to correlate the epizootic with the epidemic. He drew up curves representing the incidence of rat and human plague for each district of the city, and came to the conclusion that the epidemic begins a fortnight later than the epizootic, the climax of the epidemic being reached a fortnight later than that of the epizootic. According to Hunter the epizootic in Hongkong maintains a low level after the disappearance of the epidemic.

*Japan.* Ogata (1897) from a study of an outbreak in Formosa considered that plague might be primarily a rat disease causing a subsequent spread to man. He noticed that the rats always sickened and died before men were attacked.

Kitasato, Takaki, Shiga and Moriya (1900) were entrusted with the investigation of the outbreak in Kobe and Osaka. These observers ascertained that the rats were the first to sicken and die, the rat mortality being followed at a later period by the epidemic. Kitasato (1906) makes the statement that in Japan the epidemics have been traced invariably to rats, and that the number of rats found on examination to be plague infected runs parallel with the number of plague patients. He asserts that rats were the first to become infected and that by the time the first human cases were discovered the epizootic had assumed a well advanced form.

*Africa.* Within recent years epidemics have been investigated in several places in Africa.

Blackmore (1902) inquired into the outbreak in Port Elizabeth in 1901. He fully recognised the importance of rats in spreading plague.

Hill (1904) from observations of the epizootic and epidemic in Natal maintained that the most important agency in the spread among men was the rat.

Pakes (1904) reported on an outbreak of plague in Johannesburg.

The great majority of the 65 cases which occurred during the first 8 days were of the pneumonic type and concentrated about one place. The author believes that the origin of the first pneumonic case was not due to rats, though he is careful to point out that a rat epizootic was known to have existed in Johannesburg in the previous year. In the next 15 weeks there was a preponderance of the bubonic type amongst 48 scattered cases; this portion of the epidemic was associated with an epizootic amongst the rats.

Mitchell (1906) reviewed the principal facts which came to light in the epidemic in Cape Colony. He argued that a close association exists between the epidemic and epizootic and pointed out that when human cases gave the first intimation of infection evidence of antecedent rat mortality had as a rule been found.

Vassal (1906) noted that the rat epizootic in Mauritius preceded the epidemic by an interval of 2 to 4 weeks, and that the latter in some localities did not cease till 6 to 8 weeks after the complete disappearance of the epizootic.

*Egypt.* Gotschlich (1900) in an account of the outbreak in Alexandria in 1899 stated his belief that rats are of very variable importance in the spread of plague. He thinks that human plague may exist in the absence of rat plague and *vice versâ*, but considers that rats are important means of introducing the disease from an original focus into a new locality.

Bitter (1903) drew certain conclusions from a study of plague in Egypt during four years. He stated that it could be affirmed almost with certainty that the commencement of the epidemic is always associated with infection in rats.

*Odessa.* The observers of the limited epidemic in Odessa in 1901—1902 (Rabinowitsch and Kempner, Skschivan, Wernitz) were unanimous in attributing to rats the principal cause of the outbreak.

*South America.* Agote and Medina (1901) investigated an outbreak of plague in Asuncion, Rosario and Buenos Ayres. These authors state that their researches could not be more conclusive as to the precedence of human plague by dead rats. In their view rodents are a means of propagation of the first importance.

Artola, Arce and Lavoreria (1903) give a good account of an outbreak of plague in Callao and Pisco near Lima. They observed an epizootic amongst rats before the appearance of plague in man. Many rats sick or dead of plague were obtained in the mill of Santa Rosa, and following this epizootic 10 out of 70 workmen in the mill took plague.

*Britain.* In October 1901, 3 certain, and 5 more probable, cases of plague were discovered in Liverpool. No plague-infected rats were found in connection with any of the cases, although a good search appears to have been made. Thomson (1905) in a contribution to the subject of the relation of shipborne plague to rats based upon an analysis of records of instances of this kind arrives at the conclusion that the part played by the rat in the transmission of plague to man although real falls far short of the importance generally attributed to it.

Power (1902), in summing up similar evidence derived from records of plague outbreaks throughout the world from 1898 to 1901 compiled by Bruce Low, submits that the information thus derived goes far to confirm the belief that man and the rat are reciprocally infective, but fails to afford sufficient data for determining the degree to which man is in danger through the rat.

## 2. *The Mode of Infection of Rats in Nature.*

*India.* Simond when promulgating the theory that parasites convey the disease to man advanced the view that parasites also convey infection from rat to rat.

The German Plague Commission ascertained that rats die of plague if they eat rats dead of the infection, and put forward the suggestion that this is the method of spread amongst them in nature.

The Austrian Plague Commission (1900) acknowledged that the number (eight) of naturally infected plague rats examined by them was too small to allow of a definite opinion from the post-mortem findings, but thought that an intestinal infection was the most probable method. Neck buboes occurring in two of the rats are interpreted by them as being due to a mouth or nose infection.

The Indian Plague Commission discussed the matter at some length. They conjectured that rats might occasionally contract plague by feeding on the dead bodies of plague rats or on grain or other food contaminated by plague excretions, but influenced by certain experiments relating to feeding they believed that infection through the alimentary canal could not be a very common occurrence. Nasal infection they considered the most likely mode in cases where an epizootic is traceable to the importation of infected clothing.

Liston (1904) argued that, while rats could be infected by feeding on grossly infected food, yet they could without harm eat food which contained only small quantities of plague germs, such as might be

expected to occur in the excreta of plague-sick animals and man. Healthy animals could live in the same cage with infected animals without harm if fleas were excluded. While this was the case very minute quantities of plague germs introduced under the skin brought about infection. The flea to this worker appeared to be the most likely agent in nature to bring about infection in this way.

Browning-Smith has suggested that the excreta of plague patients may infect rats through the nose, and that an epizootic may be due either to rats eating infected food or to cannibalism, but he regards the flea as the principal agent in the spread of the disease amongst rats.

*Australia.* Tidswell (1901) stated that his experiments seemed to indicate that the disease might spread from rat to rat by inoculation through wounds from infected teeth or claws, by sharp points of bone in their food, by vermin or by feeding upon the viscera of infected animals.

Baxter-Tyrie thought it highly probable that infection from rat to rat is mainly by the agency of food infected by the excreta or saliva of plague-infected rats.

*Hongkong.* Yersin (1897) discovered in the soil of an infected house a bacillus like *B. pestis* but without virulence. He conceived that rats might become infected from such infected soil if circumstances were favourable.

Hunter is of opinion that the usual mode of infection of rats in nature is *per os*. This infection may be derived from the excreta of man and of rats, from infected clothing or from food contaminated by cockroaches, ants or flies. He draws the conclusion that the part played by fleas would appear to be over-estimated.

*Japan.* Kitasato and his colleagues (1900) regarded the mode of spread as being due to infection brought about by rats eating their infected companions. In a later contribution (1906) Kitasato notes that an infection of the submaxillary and cervical glands occurred in naturally infected rats, and infers from this that the rat becomes infected through the mucous membrane of the mouth and throat.

*Africa.* Mitchell makes no definite statement as to the mode of infection, but considers that it is unlikely that infection is conveyed to the rat by man.

Bitter (1903) ascribes the spread of the epizootic in rats to their habit of cannibalism.

*General.* Kolle (1901), Dicudonné (1903) and Kister and Schumacher (1905) favoured the view that the cannibalistic habit accounts for the



spread of infection from rat to rat. All these authors failed to find sufficient reason for the belief that insects play any part.

Finally Klein (1904) infers that the spread from rat to rat in nature by means of the flea can at best be of rare occurrence. He thinks that infected rats may convey the disease by biting their companions, and further suggests that rats become infected by plague bacilli in the faeces of infected rats gaining entrance through abrasions in the skin of other rats. Klein (1906) has recently made a further contribution to the question of the transmission of plague in the rat. He believes that certain "feeding" experiments which he carried out strongly suggest that the excreta of a plague rat, becoming dried and mixed with foodstuffs, may start an extensive infection of rats feeding on these substances. He also reviews the question of the possible transmission by the flea, and in conclusion reiterates the view already expressed by him in a former paper.

### 3. *On the Course taken by the Epizootic.*

Observations bearing upon this point which have been recorded are few in number.

Ashburton Thompson gives particulars of several instances where a watch was kept on the progress of the outbreak amongst rats living in certain buildings. His experience has been that although plague occasionally destroys practically the whole of a limited community of rats, *e.g.* those inhabiting a building, yet much more frequently it follows a slow course in such cases. Thompson further directs attention to the fact that in Sydney infection attached to localities and spread to others adjoining and contiguous with that in which it was first manifested. It appeared to be transported mechanically from an existent focus to a considerable distance, there initiating an independent focus. It is obvious from the context that Thompson believes this phenomenon to be due entirely to the agency of the rat.

Without offering any explanation of the fact the Indian Plague Commission noted that the most striking characteristic in the spread of plague through a place is its slow and steady advance from one group of houses to another and its long persistence in a quarter that has once become infected.

Hill described the course of the epizootic in Natal as a "continuous forward progression" with occasional branching offshoots and now and again a retrogression.

Gamaleia (1902) referring to the outbreak in Odessa remarked that



the plague infection in the sewer rats of the city was characterised by a complete localisation in the form of sharply circumscribed foci, there being no tendency to extension from these foci.

4. *The Natural History of the Rat in its Relation to the Epizootic.*

Observations relating to the natural history of rats, especially in so far as they may possess a bearing upon the course of the epizootic, are exceedingly scanty.

*Species.* Tiraboschi (1904 A.) has justly pointed out that most observers of the epidemiology of plague have taken no care in determining the species of rats which succumbed to the infection, since they group the affected rodents in the phrase "rats and mice." This author suggests that *Mus decumanus* and *M. rattus* are able to play a part of equal importance in the spread of plague, according as each preponderates, e.g. *M. decumanus* in large cities and *M. rattus* in ships. Some writers, e.g. Gamaleia, have asked if the natural resistance of *M. decumanus* to plague and its actual predominance in Europe are not sufficient to explain the present immunity of Europe to plague. Tiraboschi, however, from experiments(?) concludes that it cannot be affirmed with certainty that *M. decumanus* is less susceptible to plague than *M. rattus*.

Liston (1904) pointed out that while both *M. rattus* and *M. decumanus* were alike very susceptible to plague the importance of species in relation to the epidemic lay in the habits of the different species, *M. rattus* being essentially more domesticated than *M. decumanus*.

Hossack (1906) states that the rats most frequently found in Calcutta belong to the species *M. decumanus*, *Nesokia bengalensis* (a species of small bandicoot) and *M. rattus*. In the northern quarter of the city—a notorious centre of plague—where there are numerous grain godowns the *Nesokia bengalensis* accounts for 60—80 % of the total rat population. Hossack concludes that this is the rat most intimately concerned with the spread of plague in Calcutta.

Ashburton Thompson found that in Sydney during the epizootic period in 1905 0·79 % of *M. decumanus*, 0·88 % of *M. rattus* and 0·13 % of *M. musculus* were infected, the numbers being calculated as a percentage on the total number of each species examined. These numbers are in practically the same proportions as in former epidemics in Sydney.

In the Sydney report (1906) an interesting account of a small out-

break (13 plague cases) at Ulmarra is given. One hundred and six plague-infected *M. decumanus* were found and three infected mice. No plague-infected *M. rattus* was discovered. Of 1128 rats examined 1125 belonged to the species *M. decumanus* and only three were *M. rattus*.

In Thompson's experience *M. decumanus*, *M. rattus*, *M. alexandrinus* *rufus* and *M. musculus* are all liable to plague infection and all may be associated with plague in man. This observer believes that *M. decumanus* can by itself give rise to plague in man, and quotes in support of this view the case of the troopship "Antillean" which carried no other species.

Baxter-Tyrie in Brisbane also noted that plague may occur in *M. decumanus*, *M. rattus*, *M. alexandrinus* and *M. musculus*.

Nime (1904) states that *M. rattus* was the species found most frequently infected by plague in the epidemic in Formosa in the year 1896.

Kitasato (1906) asserts that the *species* of rat (*M. decumanus* and *M. rattus*) has been of very little importance in relation to the spread of plague in Japan. He appears to consider that the most prevalent rat in Japan is a race which is a mixture of *M. decumanus* and *M. rattus*.

Skschivan (1903) diagnosed 32 plague rats during an extensive examination of these animals in Odessa, from 1st. Nov. 1901 to 31st March, 1902. Of these rats one was a *M. rattus*, three were *M. alexandrinus* and 28 belonged to the species *M. decumanus*. Wernitz and Skschivan (1903) found 14 *M. decumanus* dead of plague in the cellar of a house in Odessa in which a plague case occurred in October, 1901.

In Glasgow<sup>1</sup> during the examination of rats for plague in connection with a limited epidemic about 150 were found infected. The great majority were *M. decumanus*, but a few which were obtained near the harbour belonged to the species *M. rattus*.

*Breeding.* Remarkably few observations have been made on this subject. Ashburton Thompson is of opinion that in Sydney rats breed all the year round, although probably a little less freely in the four colder months.

Gotschlich (1903) in 1901—1902 carried out in Alexandria observations on a large scale to test this point. The interpretation placed by this writer upon the results of these observations will be referred to later. From the figures given there can be no doubt that a distinct

<sup>1</sup> Verbal communication by Dr R. M. Buchanan.

breeding season of rats occurs in Alexandria, the largest number of pregnant females having been found in May and the first half of June.

*Migration.* The question of the migration of rats is an important one in its relation to the epizootic. We have met with comparatively few careful records of observations bearing on the point, but it would seem that migration of communities of rats does occasionally take place. Thus Bannerman mentions that Veterinary-Colonel Brodie Mills, Principal of the Bombay Veterinary College, on one occasion observed a migration of numerous rats from his bungalow. In this case the migration appeared to have no connection with an epizootic. Colonel Weir observed a similar migration of rats through his house near Bombay.

*The rat flea.* In a previous volume of these reports an historical sketch of the experimental work dealing with the rat flea has been given. In the present account reference has already been made and will continue to be made as occasion arises to the epidemiological side of the question.

### III. THE MODE OF ENTRANCE OF THE PLAGUE BACILLUS INTO THE HUMAN ORGANISM.

The opinions held on this subject by epidemiologists are coloured to no small extent by the views they may happen to entertain regarding the relation of the epizootic to the epidemic. It will be convenient, therefore, at this point to summarise the principal conclusions to which each observer has been led by his experience of the disease.

*India.* Bitter (1897) a member of the Commission sent from Egypt to Bombay to study plague reported that septicaemic cases played a very considerable part in the propagation of the disease. He believed that the most important factor in its spread was to be found in pneumonic cases, which in his view were much more frequent than was generally thought. The danger in these cases lay in the infectivity of the sputum. Bitter satisfied himself that infection through the skin was by far the most frequent occurrence in bubonic and septicaemic cases. He considered it possible that bacilli in the excreta of infected men and rats, and especially the sputum in pneumonic cases, might gain entrance through abrasions of the skin.

Wyssokowitz and Zabolotny (1897), members of the Russian Commission, carried out in Bombay some experimental work on monkeys with a view to throwing light upon the problem. They

showed that an infection by way of the skin and lymphatics with the formation of a bubo in the corresponding glands could be produced, although there was no evidence of a local lesion. They inferred, therefore, that the method of infection in man also involved the skin and lymphatics.

Simond from his observations in India in 1897 arrived at the idea that plague was transferred from rat to man by means of fleas. He considered that such a parasitic transmission of the plague bacillus explained most of the difficulties in the epidemiology of plague.

Hankin (1898) suggested that an intermediary insect was necessary to communicate the disease to man. He contended that plague was not conveyed to man directly to any appreciable extent by the dejecta of infected rats.

The German Plague Commission did not regard the bites of fleas as a probable means of transmission.

The Austrian Plague Commission devoted the time at their disposal for the study of the disease in Bombay for the most part to an exhaustive investigation of the pathology of human plague.

The conclusions at which they arrived from this work and from experimental work on animals may be given in detail, since they have a most important bearing on the point under discussion. They may be stated as follows—Undoubtedly in the overwhelming majority of cases infection occurs through the skin. A primary blood infection does not take place. The plague bacilli are always taken up in the first instance by the glands where they remain localised till their multiplication is so great that they invade the circulation. Minute injuries to the skin are sufficient to permit of the entrance of infection. Infection may also gain entrance by the mucous membranes of the mouth, nose and throat, the tonsils and the conjunctivae. In no single instance did anything point to a primary intestinal infection.

The members of this Commission suggested that violent scratching of an itching part could, under certain circumstances, lead to infection.

The Indian Plague Commission arrived at the conclusion that infection was as a rule by way of the cutaneous surface and that suctorial insects do not come under consideration as a means of infection.

Liston (1905) brought forward much new and valuable evidence in favour of the view that the rat flea is the transmitter of infection to man. He carried out investigations in plague-infected houses and premises from the point of view of the presence in them of rat fleas.



By the ingenious use of guinea-pigs as traps for rat fleas in houses Liston introduced a method<sup>1</sup> which has subsequently proved of great value and importance, since it affords a ready means of giving a rough estimate of the flea infestation of houses and because fleas may easily be obtained in this way for further examination in the laboratory, *e.g.* with the view of testing their capacity for infecting susceptible experimental animals. Liston further showed that the rat flea of India (*P. cheopis*) on occasion attacks man.

*Australia.* Ashburton Thompson (1900) inclined from purely epidemiological considerations to the view advanced by Simond, namely, the transmission of infection from the rat to man by the flea. Later (1906) from an analysis of the facts which he had collected during the outbreaks of plague at Sydney he came to the conclusion that the intermediary necessary to communicate the infection from the rat to man must be the "flea in one or more of its many species."

Baxter-Tyrie states that his experience points to the conclusion that the relation of fleas to the transmission of the disease has been over-estimated. He makes the conjecture that the source of infection in man, when glandular symptoms are absent or are secondary to an infection of the blood, is to be found in food which has been contaminated by the excreta or saliva of infected rats. He indeed suspects that this may hold good in many cases where apparently there is only glandular infection.

*Hongkong.* Yersin (1897) expressed the view that man becomes infected either by wounds on the skin or by the intestinal canal.

Wilm (1897) strongly urged that infection by way of the alimentary canal was a very common mode of infection.

Hunter concludes that plague-infected fleas are of no practical importance in the spread of plague, and that indeed the importance attached to skin infection in plague has been exaggerated. The principal part played by insects is the infection caused by them of food; cockroaches, flies and other non-suctorial insects are important in this respect. Hunter, further, declares that plague rats scatter plague bacilli broadcast in their excreta, thereby rendering possible a great infection of food and water. This author believes that the intestine is the principal avenue of infection in man, and that buboes are usually secondary to the blood infection which supervenes.

Hunter's views generally are supported by Simpson, who remarks: "The facility with which the lower animals contract plague by feeding

<sup>1</sup> See Historical Introduction in previous volume of these reports (vol. vi. p. 430).



is in favour of man contracting it often in the same way." Simpson makes the further statement that septicaemic cases are dangerously infective on account of the presence of plague bacilli in the excreta.

*Japan.* Ogata (1897) suggested from epidemiological considerations that plague was mostly conveyed by suctorial insects such as mosquitoes and fleas.

Yamagiwa (1897) from evidence derived from a research into the pathology of human plague concluded that a skin infection occurred almost exclusively.

Kitasato (1906) appears to believe that direct contagion from man to man plays a considerable part in the spread of plague. He suggests that the soil becomes infected by the excreta of plague rats and that soil thus infected constitutes a danger in certain instances, *e.g.* in children.

*Africa.* Blackmore regards the rat flea as the usual transmitting agent and suggests that the human flea may occasionally act as the intermediary.

Hill came to no definite conclusion with regard to the rôle of fleas. He contends that no grounds exist for assuming the agency of fleas in the cases without buboes, and invokes as an explanation of such cases an infection through either the mouth or nose or by particulate matter in air or in food.

Bitter (1903) does not consider that blood-sucking insects play in themselves an important part in the transmission of plague.

In a report on an outbreak of plague in Mauritius (1899) it is stated that inoculation by the flea certainly did not hold good in the majority of cases.

*General.* Klein entertains the idea that man may contract plague through skin abrasions by the faeces of plague-infected rats. By comparison of cultural and animal tests of several strains of *B. pestis* obtained from different sources he professes to distinguish between two types of the bacillus, (1) a "rat" type, a weakly virulent bacillus regarded by him provisionally as that proper to the rat, and (2) a virulent "human" type which may occur in the rat, but by assumption occurs less commonly than the "rat" type.

Dieudonné (1903) makes the surmise that the bacilli in the excreta of septicaemic cases enter through very small abrasions of the skin. He considers that fleas, flies and bugs may have a certain importance, because in the puncture or "finger scratch" plague bacilli adhering to their bodies may be rubbed in. He thinks, however, that the danger from fleas is inconsiderable.

#### IV. THE MODE OF SPREAD OF THE INFECTION.

Having epitomised the views held by epidemiologists upon the question as to how the plague bacillus gains an entrance into the human organism, we may proceed to chronicle the various points of view on the wider issue which concerns the means by which the infection of plague is spread. It will be convenient to arrange the subject under four headings:—(1) the possibility of infection by direct contact with the patient suffering from plague; (2) the question of the infectivity of houses; (3) the spread of infection by indirect means, *e.g.* through the medium of infected clothes or articles of merchandise; and (4) the importation of infection into a hitherto uninfected locality.

##### 1. *Infection by Direct Contact with a Patient suffering from Plague.*

*India.* Bitter (1897) from his experience in Bombay reported that intimate contact with the sick was the cause of the majority of the cases.

Simond, on the other hand, arrived at the opinion that infection from man to man plays only a secondary rôle in the propagation of plague.

The Indian Plague Commission reviewed the bacteriological evidence bearing on the infectivity of patients suffering from bubonic or septicaemic plague as follows. The ordinary bubonic case may be considered as non-infective until the septicaemic symptoms supervene; from that time onwards the plague patient will become infective by the fact that the plague bacilli escape from the body in the discharges; on the one hand from the nose, lungs and intestinal tract, and on the other hand from the kidneys. In cases which recover a further possibility of infection will be afforded by the fact that the pus from the suppurating bubo, as well as the sputum and saliva, may contain the infective agent.

Bannerman refers to the well recognised experience in India that attendants in hospitals remain singularly free from danger of infection.

The experience of Captain Thomson, I.M.S. (1907), who had charge of a large plague hospital in Bombay, is worth quoting in this connection. Although 533 cases of plague were treated in his hospital, there were no instances of the spread of plague from the patients to the nurses or attendants. "In upwards of 240 instances the friends of the patients attended their sick and in 20 instances scarcely ever left their bedside, and in not a single case did the disease spread to the friends."

Dr Dallas, who also had a large hospital experience in Bombay, has stated that though there were "about 400 people—men, women and children—who either visited their sick friends or remained constantly at their bedsides, together with the cases under observation, in not a single instance did any of these persons contract plague."

Bannerman further cites instances reported by Sanitary Officers in India where in a village people of different castes, and having no intercourse with each other for this reason, were affected, the incidence of cases amongst them giving no support to a theory of direct contagion, and being explicable only on the view of a common source of infection.

Evidence of a similar character has been brought forward by Browning-Smith. This observer states that in the Punjab villages infection often spreads from one house to a contiguous one placed back to back, although the entrance doors are widely separated by actual walking distance and although the families living in each house are absolutely prevented by caste rules from having any relations with each other. In such an instance, however, the rats in each house may freely communicate by means of rat holes and burrows.

*Australia.* Ashburton Thompson sums up his experience in Sydney in this manner :—The disease was not directly communicated from the sick to the well. Cases in an infected locality occurred irregularly, and the infection showed no special tendency to attack adjoining houses. Secondary cases rarely happened in a building. Plague in short owes nothing of its epidemic form to communication with the sick.

*Hongkong.* Lowson (1895) reported that no attendant in the plague hospital contracted the disease.

*Japan.* Kitasato (1906) appears to suggest that direct contagion from man to man frequently takes place.

*Africa.* Blackmore stated that in the outbreak in Port Elizabeth in no case was there direct evidence of man to man infection and in most cases the possibility of this was definitely excluded.

Hill considered that in Natal the disease had but a very slight tendency to spread from man to man even when the lungs were affected, except in the case of acute primary pneumonia.

It seemed to Mitchell that the bubonic form of plague in Cape Colony was only slightly infectious.

Bitter (1903) remarked that the transmission of plague from man to man had not played an appreciable part in Egypt, but he adhered to the view that such a method of transmission was really the cause of the epidemics in India.

*South America.* Artola (1903) could find no proof of direct transmission of plague from man to man in the outbreak at Callao already referred to.

*Europe.* Lastly, Rabinowitsch and Kempner asserted that not a single case at Odessa could be traced to the transference of infection from man to man.

## 2. *The Infectivity of Houses.*

*India.* The German Plague Commission were persuaded that with relatively few exceptions infection takes place within the house.

The Indian Plague Commission called attention to the general experience of observers in India with regard to this question, namely, that plague is essentially a disease of locality. The commissioners gave credence to the view, that this circumstance was best explained by a contamination of clothes and other effects by excreta of plague-infected men and rats.

Hankin (1905) considered that in a town threatened with plague the grain dépôts and all industries attracting rats ought to be regarded as dangerous, and pointed out that in India it has been found that the infected locality is a far greater source of danger than the plague patient. He criticised the view that plague infection in a house is due to bacilli from the dejecta of the patients, on the ground that it was inadequate to explain cases of infection of contacts 20 days to four months after the arrival of the first patient.

The belief that the infection of plague resides in the soil has been entertained and advocated by Creighton (1905) who adopted it in his *History of Epidemics in Britain* (1891) and reaffirmed it in 1905 after having paid a visit of investigation to India. He thought the infection rose to the interior of dwellings with the ground air and was commonly taken in by man with the breath.

Bannerman points out that in India voluntary evacuation of infected localities is widely practised, for no other apparent reason than that the people believe the infection to reside within the houses. He further cites instances showing that a premature return to an infected house has proved dangerous.

*Australia.* Ashburton Thompson gives it as the experience in Sydney that the infection of man is always contingent on his presence in buildings of some sort, and that, moreover, the incidence of infection on the houses is erratic. Out of 221 plague houses in 1900 as many as 215 escaped in 1902. The source of infection in a considerable



proportion of the cases was traced to produce stores and other similar buildings, which offered great attraction for rats.

In Brisbane, Baxter-Tyrie also found that a heavy incidence of plague rats and cases was associated with produce stores.

*Africa.* Hill in Natal states that 22% out of a total of 221 cases were employed in produce stores or stables.

*Hongkong.* Reference has already been made to the bacillus which Yersin found in an infected house in Hongkong and which he evidently believed to be a modified *B. pestis*. So far as we are aware this observation has never been repeated.

Simpson (1904) came to the conclusion that to infected houses was principally due the maintenance of plague in an infected locality, and he believed that the infection adheres to those houses which are old, dark, damp and rat ridden.

Discussion of a vague character has occasionally been made as to the recurrence of plague cases within the same house year after year. The point seems to us to require further investigation since no instance of this kind has been put forward which will bear strict scrutiny.

### 3. *The Spread of Infection by Indirect Means, e.g. by infected Clothing, Food, or Articles of Merchandise.*

*India.* Bitter (1897) entertained the idea that articles which may have been infected by the sick person—clothing, linen, carpets, etc.—were likely to convey infection.

Hankin (1898) remarked on the circumstance that the infection can remain for a long time in clothes.

The Indian Plague Commission concluded that clothes and other effects soiled by excretions of plague-infected men and rats are infective, and may remain infective for very considerable periods.

*Australia.* Ashburton Thompson believed that the infection in the Sydney epidemics was not communicated in any important degree from the sick to the well by indirect means.

*Hongkong.* Wiln held the belief strongly that contaminated food was chiefly responsible for spreading infection. This view is also advocated by Hunter.

*Africa.* Hill attributed the source of infection in eight cases out of 221 in the Natal outbreak to "fomites." He thought there was no evidence for the view that infection may be conveyed through food.



Mitchell imputed the infection of six out of 337 cases in Port Elizabeth to infected clothing and other articles.

Gotschlich (1900) believed that an indirect infection through the medium of houses or clothes and other articles contaminated, *e.g.* by sputum, was an important means of spread. This opinion has also been expressed by Diendoné.

4. *The Importation of Infection into a hitherto uninfected Locality.*

*India.* Simond stated that the introduction of plague rats into a hitherto healthy place was generally followed after a brief interval by epidemic cases in man.

Hankin (1905) thinks there can be no doubt that plague is not infrequently carried from place to place by persons who themselves escape or are not the first attacked in the places to which they have carried the infection.

In the summary by Bannerman, from which we have already quoted, several instances are given in which clothes removed from a plague house to a hitherto uninfected house infected either the inhabitants in this house or the rats. Bannerman sums up the evidence bearing on the question of the transportation of infection and its introduction into a fresh locality as follows:—

It seems certain that human beings are the carriers of infection when it is introduced into a new area. The man who introduces the infection may not be the first victim and may not develop the disease. It seems to be more common for infection to spread amongst rats in the house where the introducer lodges so that in this way an epizootic breaks out and is followed by the epidemic.

With regard to the possibility of transportation of infection by means of grain or merchandise Bannerman, from a *résumé* of opinions held by authorities in India, concludes that grain cannot be incriminated.

Liston (1905) believed that infection could be conveyed from one place to another, either by infected rats and fleas transported by ships and trains conveying merchandise or by infected fleas carried on the clothing of man. He was unable to communicate the disease to animals by means of soiled clothing and contaminated food.

Browning-Smith thinks that the spread of plague from one locality to another is generally due directly to human agency, and that plague infection may be transported for long distances by any articles

contaminated with infective material or harbouring infected fleas or concealing the dead bodies of plague rats.

*Australia.* Ashburton Thompson believes the successive epidemics at Sydney to have been due to repeated infection from importation by sea of infected produce. He regards "produce of all sorts, returned empties with packing still in them, and bundles of empty bags" as the most dangerous class of goods concerned in the transportation of plague infection.

*Hongkong.* Wilm considered that the contagion of plague might be imported into a fresh locality by men suffering from the disease, by fomites or by animals. Simpson thought it possible that infected food might be imported into the colony.

*Japan.* Kitasato (1906) recognises two modes of importation into a new locality: (1) contagion from imported plague patients; (2) contact with disease germs mingled with the freight brought in from some infected region. Rats are the first infected by such an imported infection, and by the time the first human victims are discovered the epizootic has assumed a well advanced form.

*Africa.* Lastly, Mitchell believed that rats had been the means of introducing plague into the ports of Cape Colony, and that they also spread infection from infected centres to other places.

## V. CERTAIN ALLEGED CONTRIBUTORY CAUSES OF THE SPREAD OF INFECTION.

Having summarised the views held as to the methods by which plague infection is considered to be spread, we may now draw attention to certain factors which have been represented as favouring the dissemination of infection in a locality. These are (1) insanitary conditions generally, and (2) the occurrence of plague in domestic and other animals.

### 1. *The Influence of Insanitary Conditions on the Spread of Plague.*

*India.* Bitter while serving on the Egyptian Plague Commission came to the conclusion that insanitary conditions aided in spreading plague, for the reason that under such conditions the danger of intimate contact with the sick is increased.

Hankin (1898) took quite the opposite view. He thought that the evidence available at the time reduced to the rank of an unnecessary hypothesis the influence of those insanitary conditions which were pre-

sumed to favour the spread of plague. Badly constructed houses, according to his view, were more exposed to contagion not for this reason but because they offered shelter to rats.

The German Plague Commission appeared to consider that insanitary conditions, such as dark, badly ventilated and overcrowded dwellings, were favouring circumstances for the spread of infection, and that when such conditions are present an epizootic amongst rats is not a necessary concomitant of the epidemic.

A majority of the members of the Indian Plague Commission stated that they were unable to find any evidence which went to prove that the ordinary sanitary defects exercised any marked favouring influence on the spread of plague. They considered overcrowding to be the principal defect.

The President of the Commission, Sir T. Fraser, dissented from this view. He gave as his opinion that after plague has been introduced into a place its extension and virulence are chiefly fostered by the pollution of the atmosphere and other conditions which result from the inadequacy of ventilation and sunlight and from the uncleanness within and near dwellings, which characterise the great majority of native houses.

Recent opinion in India appears to be divided on the subject, judging from the account given in Bannerman's article.

*Hongkong.* The majority of the observers in Hongkong (Lowson, Atkinson, Pearse and Simpson) agree in regarding general insanitary conditions as important contributory causes of the spread of the epidemic. Simpson considered that the insufficient latrine accommodation and the insanitary condition of many of the existing latrines probably favoured the endemicity of plague in Hongkong.

*Africa.* Mitchell believed that sanitary defects undoubtedly had their effect in fostering plague in Cape Colony.

*General.* Hope (1902) remarks on the circumstance that the cases in the limited outbreak at Liverpool were entirely dissociated from the squalor and filth with which plague is commonly found. Dicudonné looks upon plague principally as a disease of filth and poverty, and remarks that where light and air are plentiful and cleanness reigns plague finds no settlement.

2. *On the occurrence of plague in domestic and other animals.*

Undoubtedly the rat is not the only animal apart from man which is subject to plague in an epizootic form, but it is not our intention to collect instances of epizootics of this kind. No one will, we imagine, seek to controvert the statement that no animal approaches the rat in importance from an epidemiological standpoint. It is, however, beyond question that outbreaks in mice are not infrequently to be found concurrently with the rat epizootic. Several examples of this have already been given. Mitchell signals the fact, but remarks that although plague-infected mice were frequently found they did not appear to play any important part in the spread of the disease in Cape Colony.

The statement has been made that certain domestic animals are liable to plague infection during an epidemic and that they constitute a grave danger to man when thus infected.

Wilm (1896) asserted that fowls contracted plague by eating infected material. He made the further assertion that in 1896 a shipload of pigs imported from an infected locality into Hongkong were proved by bacteriological evidence to have died of plague. Atkinson, Pearse and Hunter (1903) stated that poultry from the markets in Hongkong were proved to have died of plague.

Hunter (1904) notes the following animals as having been found affected with spontaneous epizootic plague:—rat, mouse, cat, guinea-pig, monkey, hen, pigeon, turkey, goose and duck. He also makes the statement that plague has been experimentally given to the following animals:—pigs, calves, sheep, monkeys, hens, pigeons, turkeys, geese, and ducks.

Experiments on a large scale were carried out in Hongkong in 1902 by Simpson assisted chiefly by Hunter. Simpson (1905) has summarised the results of these experiments thus:—

“From the experiments it is shown:—

(i) That pigs, poultry, and cattle are susceptible to plague whether derived from the infection of a human being infected with plague, or from their own species, or from some other animal.

Sheep are also susceptible.

(ii) That plague among animals may be acute and rapid in its termination, or chronic and slow in its course. In neither case may the symptoms be very marked.

(iii) That the animals take the infection of plague as easily by feeding with plague material as by inoculation.



(iv) That plague material from man, pigs, poultry, cattle and monkeys, will give plague to rats, and that plague material from rats will give plague to monkeys by feeding, by inoculation, by contact and without contact; and if to monkeys probably to man by the same channels."

Pearse in his report (1904) has criticised Simpson's experiments in detail, and throws some doubt upon the significance of his observations.

Bannerman repeated these experiments in Bombay but with negative results. Hill also attempted to repeat them in Natal with fowls, pigs and calves, but his experiments were uniformly unsuccessful.

Simpson (1905) makes the following observations on the results of the Natal experiments:—

- (i) The experiments were not carried out in the epidemic season.
- (ii) The strain of bacillus in the Natal epidemic may not have possessed that degree of virulence which belongs to the bacillus in China.
- (iii) There may be a more or less comparative racial immunity among animals in one country as compared to another.

## VI. THE SEASONAL PREVALENCE OF PLAGUE.

The last problem with which we have to deal in this account relates to the peculiarity which has long been recognised of the marked prevalence of plague in a particular place at a certain definite season of the year. At or about the same date plague yearly reappears, rises, declines and disappears.

This prevalence varies sometimes in a striking manner in different places, and even in places not far distant from each other. As an example Bombay and Poona may be cited. These places are situated only about 80 miles apart, and yet the plague season of one may be said roughly to correspond to the off plague season of the other.

The reason for this seasonal prevalence has been much speculated upon by epidemiologists, but it is apparent from a review of the literature that very few facts have been brought forward which will serve even partially to explain the phenomenon. It may be of interest, however, to summarise the observations which have been made upon the subject.

*India.* Simond suggested that retention of infection by fleas may be the cause of the recrudescence which usually occurs a year after the first appearance of the outbreak. He, further, believed that variations in the rat population is an essential cause, although not the only one,



of the yearly recrudescences. Thus, he ascribed the decline and disappearance of the infection to the death of a large part of the rat population, to a migration of large numbers of rats, and to an immunity of a certain proportion of those remaining. Simond thought that the recrudescence coincided with a repopulation of the rat community by new generations of susceptible rats.

Hankin (1905) drew attention to the disappearance of dog fleas in hot weather in Agra, and suggests that if rat fleas similarly disappear this may be a possible explanation of the seasonal decline of the epidemic.

The German Plague Commission concluded that an unusually high temperature by itself could not be a determining factor in seasonal prevalence.

The Indian Plague Commission came to no definite conclusion upon the matter. They remark "If the rise and fall of plague mortality is in reality dependent upon the meteorological variations (temperature and humidity) it seems quite clear that the inter-dependence must be a very indirect one."

Browning-Smith in a recent article expressed his belief that not one but many factors must enter into the problem. Amongst these he regards a prevalence of fleas as essential. Certain subsidiary factors have an influence, *e.g.* the breeding of fresh generations of rats, temperature, humidity, and the habits and occupations of man.

*Australia.* Ashburton Thompson remarked that the epizootic and epidemic period in Sydney is the season of fleas, at least of the dog flea. Moreover, Tidswell observed that rat fleas were more abundant in the epizootic period than at the end of the epizootic.

Baxter-Tyrie states that rainfall during an epidemic was invariably followed by an increase of cases, a circumstance which he attributed to rats being driven from sewers into buildings.

*Hongkong.* Clark noted that in Hongkong the disease declined rapidly as soon as the mean weekly temperature exceeded 80° F.

Pearse thinks that in Hongkong the worst epidemic period is that in which the temperature varies from about 70°—80° F., and that with a rise to 81° F. and over the epidemic declines.

Simpson makes the statement that in this city any continuous temperature above 83° F. is followed by a fall in the number of cases and ultimately causes a cessation of the epidemic.

*Africa.* Hill in Natal thought there was no evidence to show that the outbreak bore any relation to the temperature of the air or to

rainfall. He noted that fleas on rats were scarce in Maritzburg when plague was absent.

Mitchell remarks that no definite general correlation between temperature or rainfall and the epidemic prevalence has been observed in Cape Colony.

Gotschlich (1903) carried out some interesting observations to test the statement that the recrudescence is due to a large addition of susceptible rats to the rat population, consequent on a definite breeding season. An examination was made in Alexandria of 6500 rats caught alive from the 15th August, 1901, to the same date next year. A note was made of those found pregnant and a percentage of these was calculated on the total rats during each fortnight. The results were briefly as follows. During the plague-free winter months—November to February—less than 2% of the rats were found pregnant. In March and the first half of April there was a slow rise, till in the second half of April 6% of pregnant females were found. The percentage rose to 12% in May and the first fortnight of June and then quickly fell, keeping an average of 5% till a minimum in December of under 1% was reached. The maximum period corresponded well with the summer epidemic of bubonic plague.

*General.* It is stated in a report of the outbreak in Mauritius that temperature and other climatic changes did not seem to have had any influence on the progress or otherwise of the epidemic.

Agote and Medina (1901) in South America conclude that the climatic conditions favourable to plague are a moderate temperature and a relatively low humidity of air and soil.

Finally Dieudonné remarks that meteorological conditions play no great rôle in the origin and spread of the disease, and have perhaps only an indirect influence.

#### *Chronic Plague in Rats.*

Before bringing this section to a close we may refer briefly to the suggestion that the infection may continue throughout the off plague season in the bodies of rats suffering from a chronic form of plague.

Simond (1898), for example, believed that after the epizootic ceased to be acute plague continued to linger amongst the rats in a benign form, and that sporadic cases in man occurring during the off season might be thus explained.

Kolle and Martini (1902) described a chronic form of plague in rats which had been experimentally infected by various methods months

previously. These rats showed cheesy submaxillary and bronchial glands and induration of the lungs; the bacilli in the lesions were virulent. They considered that these results had an important bearing upon the problem of the seasonal prevalence of plague.

Gotschlich (1903) agreed with these writers as to the significance of their observations and explained the recrudescence of the epizootic by supposing that when the rat population receives an accession to its ranks of highly susceptible young individuals infection from such a latent case might start a fresh epizootic.

Ashburton Thompson could find no evidence that plague persisted in a chronic form in the rats in Sydney after the epizootic had ceased.

We have elsewhere given an account of chronic plague in rats caught in several villages in the Punjab (vol. VI. p. 530; vol. VII. p. 457). The condition of these rats seems to be clearly different from that described by Hunter (1904), who in Hongkong found a large number of rats suffering from chronic plague. These animals were much emaciated and suffered from chronic diarrhoea; on section necrosed areas of cheesy material were found in the lymphatic glands and viscera, containing few plague bacilli but capable of giving rise to acute plague when administered to healthy rats. He found that such animals were caught more frequently in the interval between, than during, the epizootics of acute plague. Our rats on the other hand showed no signs of general illness and in only one instance was emaciation noticed.

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## XXII. THE EPIDEMIOLOGICAL OBSERVATIONS MADE BY THE COMMISSION IN BOMBAY CITY.

- I. Introduction.
- II. Description of Bombay.
- III. Methods adopted for studying the disease.
- IV. The epizootic.
  - I. The rodents of Bombay, habits and breeding season.
  - II. The rat epizootics in relation to time and place.
  - III. The relation of the decumanus epizootic to the rattus epizootic.
  - IV. Plague in other rodents.
  - V. Summary.
- V. The epidemic and its relation to the epizootics.
  - I. General account of the epidemic of 1905—1906.
  - II. The relation of the epidemic to the epizootics in time and place.
  - III. Summary.
- VI. The sanitary circumstances in Bombay City which influence the spread of epidemic plague.
- Appendix I. Tables, charts and maps of the occurrence of rat and human plague in the different sections of Bombay City.
- Appendix II. Statistical report on certain rat figures, by M. Greenwood, Esq.

### I. INTRODUCTION.

One of the first duties which the present Commission had to undertake was to decide where the epidemiological observations which were necessary to be made should be carried out. After various parts had been visited, it was decided that the headquarters of the Commission should be in Bombay, and that Bombay City and Island should, in the first instance at least, be put under observation for epidemiological purposes.

Three considerations rendered Bombay a suitable locality. In the first place Bombay City had suffered from plague since the autumn of 1896, each year seeing a fresh epidemic. The seasonal prevalence was well marked, and it was recognised that any hypothesis of the etiology of plague, if a true hypothesis, would have to explain adequately this phenomenon.

Secondly, there was already in existence in Bombay an excellent Municipal Department, with a large staff under the direction of an experienced Medical Officer of Health, Dr Turner. Long before the Commission was formed the Bombay Municipality, at the instigation of their Health Officer, had made a representation to Government, asking that a scientific Commission be appointed to investigate plague in co-operation with their Health Department. The Commission, therefore, anticipated that every assistance would be given to them by Dr Turner and his entire department, and this anticipation was soon proved to be correct.

Thirdly, Bombay possessed at Parel on the outskirts of the City a Government laboratory complete in every way and well suited for the requirements of the Commission. Through the kindness of the Director Lt. Col. W. B. Bannerman, I.M.S., accommodation was given to the Commission in its buildings, and the whole resources of the laboratory were freely placed at their disposal.

While these advantages were apparent, it was also seen that Bombay possessed several disadvantages for an epidemiological study of plague. The population is large, nearly a million, varied and scattered over a wide area. Further, it was recognised that during the plague epidemic, when from 200 to 300 cases would occur daily, it would be impossible for the members of the Commission themselves to collect detailed information concerning every plague case, and that the general density of both rat and human plague would in all probability somewhat obscure the relationship between them.

It was therefore considered desirable to choose a few isolated villages of from 1000 to 5000 inhabitants, in which plague had periodically recurred, and to endeavour to make in these places a more complete epidemiological study. Arrangements were accordingly made to undertake the study of rat and human plague in four villages on the outskirts of the city of Bombay and within easy motor distance of the Parel laboratory. It was also arranged that two villages in the Amritsar district of the Punjab should be treated in the same way, one of the members of the Commission with a separate staff being told off for this duty. It was anticipated that the general relationships of the epizootic and epidemic would be obtained from Bombay city, some errors being corrected by the very large number of plague rats and of human cases dealt with, and that the more minute details would be elucidated in some of these six villages.

We propose first to deal with Bombay and then to pass on to the villages.

## II. DESCRIPTION OF BOMBAY ISLAND AND CITY<sup>1</sup>.

(Map I.)

Bombay is a large island lying with its long axis almost due north and south.

The general shape is seen from the accompanying map. It has a length of about 10 miles and its greatest breadth is about three miles. Its total area is about 22·4 square miles. As regards the configuration of the Island it may be said in general terms that it is low lying. There are a few hills, such as Malabar Hill, Parel Hill, etc. but the greater part of the town is built on low ground, a considerable portion of which has been reclaimed in recent years from the sea.

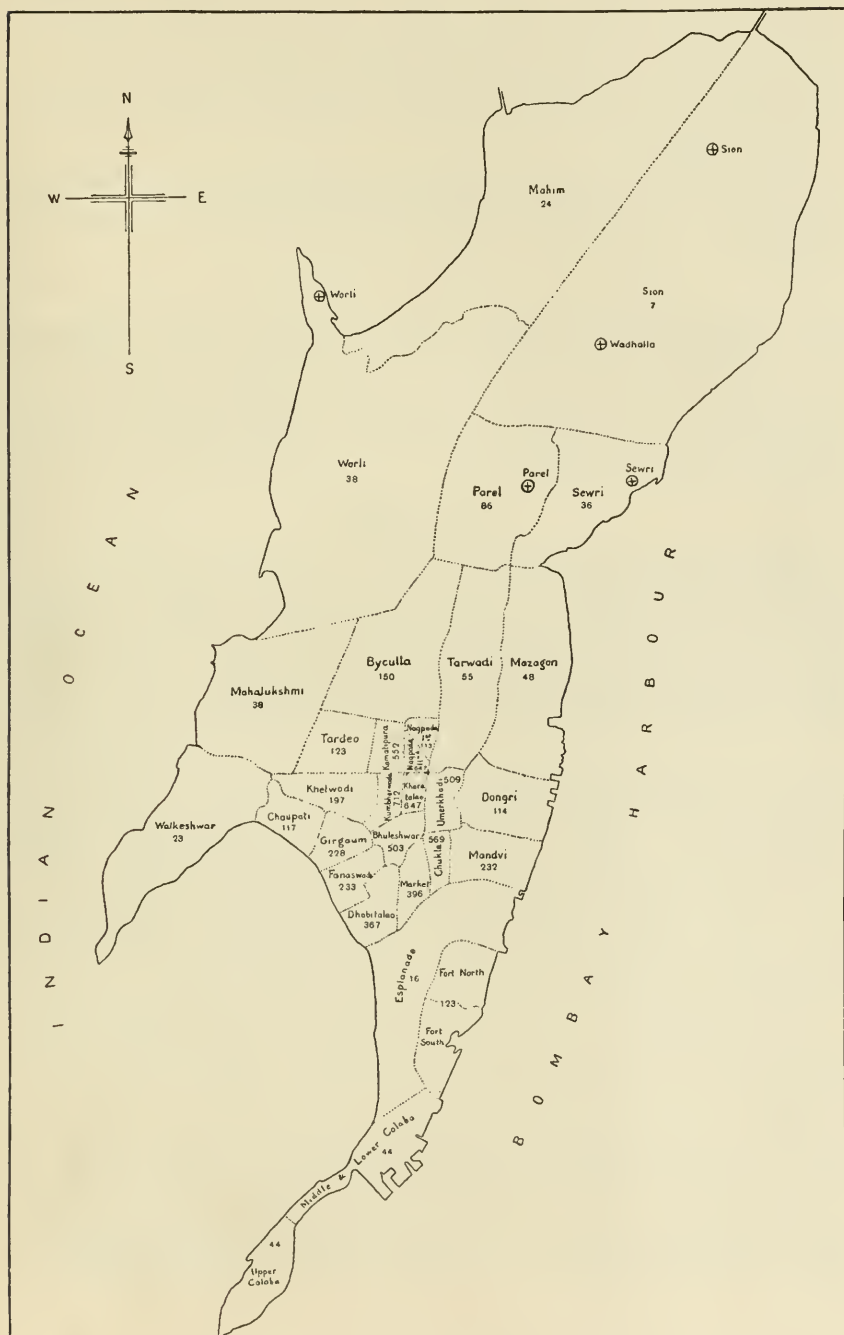
At the last census (February, 1906) the total population was found to be 977,822 which, divided over an area of 14,385·89 acres, gives a general density for the whole Island of 67·97 persons per acre. The population is however very irregularly distributed, the density varying from 7·1 per acre in one section to 711·7 per acre in another.

Lying as it does within the tropics on the coast of the Indian Ocean, Bombay comes under the influence of the seasonal monsoons. During the months of November to April the north-east monsoon blows, while from May to October the south-west monsoon prevails. In the former months there is, as a rule, no rain, while during the south-west monsoon there is an average rainfall of from 60 to 80 inches; the bulk of this falls in the months of June and July. The humidity is fairly high all the year, but is increased during the rainy season. The mean daily range of temperature is at no time large; in the winter months it is from 10° to 12° F., in the hot months about 7° or 8° F., while in the rains it is only 3° to 5° F.

In the cold season (November—March) the maximum temperature is on the average about 87° F., the minimum 65° F. and the mean 75° F. The temperature gradually increases during the months of March, April and May until at the beginning of June the maximum is on an average 90° to 95° F., the minimum about 75° F. and the mean about 85° F.

<sup>1</sup> The statistical information which we propose to give has been obtained from the Census Report of Bombay for 1906, issued by the Municipal Commissioner, and from Parts V. and VI., Vol. xi. of the report of the Census of India for 1901. These parts were compiled and written by Mr S. M. Edwardes, I.C.S. for Bombay city.

MAP I. Bombay. Town and Island showing division into sections.



The numbers show the population per acre.

The villages specially noticed are indicated ⊕.



There is a considerable fall of temperature in the rains, the mean, however, still remaining about 80° F. There is a slight rise again in October during the time the south-west monsoon is being driven back by the north-east. On the whole, therefore, the climate of Bombay may be described as hot and moist, especially during the months of May to October inclusive.

We shall have occasion to give below a more or less detailed description of the sanitary arrangements and habits of the people of Bombay.

### III. METHODS ADOPTED FOR STUDYING THE DISEASE.

It will be best to describe in two parts the methods adopted by us for studying the epidemiology of plague in Bombay city: (1) those relating to the epidemic, and (2) those relating to the epizootic amongst rats.

#### I. THE EPIDEMIC.

The arrangements for the purpose of observing the epidemic were made on a large scale, our aim, indeed, being to collect information regarding every plague case throughout a whole year. Observations were carried on during a longer period, namely, from 1st July, 1905, to 30th September, 1906, but the data presented in this report fall within the 12 months from the 1st October, 1905, to 30th September, 1906.

It would have been impossible for the members of the Commission alone to cope with the enormous amount of work involved in this attempt, but we were fortunate in being helped greatly by the Medical Officer of Health for Bombay, Dr J. A. Turner. To his cordial co-operation and ready concurrence in our suggestions the scheme owes much, for he placed at the disposal of the Commission a large part of the sanitary and medical staff of the Health Department of the City under his charge.

The following account gives a description of the sanitary organisation of the City, in so far as it is concerned with our study of the epidemic.

The City and Island of Bombay is divided into ten districts, to each of which a qualified medical practitioner—the District Registrar—is appointed. Each of these ten districts is divided for the purposes of the Health Department into two or three sections. These sections, and not the districts, are the areas, which we have chosen for

description in our account of the epidemic and epizootic in Bombay (vide Map I.).

The duties of the District Registrars are mainly in connection with the registration of the births and deaths, but they also have charge of a dispensary where poor patients are treated. In addition they attend the poorer class of patients in their homes. The District Registrars have a particularly thorough and extensive knowledge of their districts, and, moreover, their familiarity with the various languages<sup>1</sup>, habits and customs of the people gives them unrivalled advantages in obtaining as accurate information regarding the mortality from plague as is possible in a city like Bombay. It may be noted, also, that they have an unusually wide experience of the disease on its clinical side—such indeed as can only be acquired in a city affected to the extent that Bombay has been for the past ten years. Each is assisted in the general duties of his office by two sub-registrars who are usually Hospital Assistants<sup>2</sup>. Each District Registrar has also charge of a Disinfecting Staff, which is under the direct supervision of an Inspector.

The District Registrars, their sub-registrars and the Inspectors of Disinfection were entrusted with the task of collecting the information which we desired. The whole of this staff was supervised on our behalf by two selected medical officers of the Health Department of the City, Dr D. A. Turkhud and Dr J. S. Nerurkar, themselves familiar with its work and with special knowledge of the local conditions in the various districts.

Finally, the whole of the work carried out by the District Registrars and their subordinate staff was superintended daily by the members of the Commission, who personally checked the accuracy of their work.

We may now describe by what means the District Registrars become aware of the plague cases and of deaths from plague.

(1) Since in Bombay plague is a notifiable disease, medical practitioners in attendance on cases report them to the Medical Officer of Health. The information is immediately forwarded to the District Registrars, who in turn inquire personally into the cases.

(2) For each district of Bombay a considerable number of men are employed by the Health Department, whose sole duty it is to obtain information with regard to births and deaths in the district. Each man is told off to supervise a small area,

<sup>1</sup> "Some idea can be formed of the cosmopolitan character of our city and island by observing that 62 different languages or dialects are spoken within its limits." Edwardes' *Census Report*, Bombay, p. 39.

<sup>2</sup> A Hospital Assistant is a man who has undergone a three years' medical training in a Government Medical College.

so that in course of time he becomes familiar with all the inhabitants living in this area. This staff is so organised that men are on duty both by day and night. For the whole of Bombay there are 62 of these men who are styled "birth registration karkoons," and 90 styled "death registration ramosis," besides a group of 34 "death registration karkoons," the last named being on special duty at cemeteries and burning ghats. Each man in the two former groups is provided with a book of printed slips in triplicate.

When a "death registration ramosi" observes a funeral party he fills up from information supplied by it the address of the deceased person together with other particulars. He then gives one of the copies to the funeral party, by whom it must be handed over to the karkoon on duty at the cemetery before the body can be buried or burned. A second copy of the slip is sent at once to the District Registrar, who, if the case has not been already notified, makes inquiries into it by visiting the house. If the body was not examined before death by a practitioner, the District Registrar arrives at a conclusion as to the cause of death by eliciting information regarding the history and symptoms of the illness from the neighbours and from the relatives or friends.

(3) When a suffering case of plague in a large building, *e.g.* a chawl, comes to the notice of the District Registrars or of his sub-registrars, they inquire into other suspicious cases of sickness in the building. Evacuation of a tenement in a large building during the epidemic is naturally regarded by them as a suspicious circumstance, and frequently leads to the discovery of plague cases in the other parts of the building.

(4) Certain influential men in the various communities, *e.g.* Hindu or Mahomedan, have acted for years as voluntary plague workers and give valuable aid by furnishing information regarding plague cases to the registration authorities.

(5) Many cases suffering from plague are not attended by medical practitioners but by unqualified native physicians or "hakims." These cases are occasionally reported to the sub-registrars by hakims or by the neighbours. When this happens the cases are verified by the District Registrars.

We may now proceed to describe how the District Registrars and their staff were utilised by us for the purpose of collecting the necessary epidemiological data.

Printed cards with headings relating to the various points on which information was required and arranged in a convenient form were distributed amongst the District Registrars (Form I.). A printed copy of instructions for filling up these case cards was also supplied to each individual whose duty it was to help us in this direction. These cards were filled up by them on the spot when visiting a plague case.

Comment may be made on two of the headings on this card.

(1) At the outset we entertained the idea that it might be possible to trace the source of infection, if not in all the cases at least in a considerable number. Difficulties, however, soon arose in obtaining

Serial No.      District      Reporter's Name      Date      Form I.  
See also No.

Name		Age	Sex	Caste	Employment	Residence	Duration of residence	Previous residence	
Date when left work	Date of attack	Date of death	Date of disinfection	Case examined before death					Case not examined
					Buboes				
					Symptoms				
					Diagnosis				
Description of house				Contacts					
Number of rooms				Names of contacts					
Position				Cases among contacts in house or building					
Ventilation				Names of attendants					
Light				Cases among attendants					
Overcrowding				Cases among contacts at place of employment					
Floor				Migration of contacts; new address					
Ceiling									
Tiles									
Proximity to gully									
Description of building									
Number of houses in									
Number of storeys									
Number of inhabitants									
Shops on ground floor									
Condition of adjoining gullies									

(Form I: back)

### Possible sources of infection.

Information concerning rats. See opposite.

Has previous case occurred in same house or building? Reference.

### Evidence of importation of infected articles.

Evidence of attendance at funeral party or elsewhere.

Further history of case. If admitted to hospital, to which and date? Hospital No.

When and where the nearest plague-infected rat was found.

Proximity of house to grain godown, stable or other place likely to shelter rats; direct evidence as to occurrence of rats in such places.

Direct evidence as to occurrence of rats in house, *e.g.*, dung, capture by disinfecting staff, fleas, burrows, nests, etc.

Circumstances in house favouring rats.

History from inhabitants as to occurrence and time of occurrence of live or dead rats in house.



information on this point. From personal investigation of a large number of cases we became convinced, that with the conditions which exist in Bombay it was impossible to arrive at any definite conclusion as to the exact source of infection in even a small proportion of the cases. The reason for this is twofold. First, the majority of the people conceal the occurrence of dead rats in their house largely from fear that disinfection will follow the avowal of the fact, and also on account of a habit of suspicion which is characteristic of the native of India. In the second place, even when the people freely gave whatever information they possessed, it was difficult in the epidemic period to narrow down the possibilities of infection to a particular source, since the infection was widely spread all over the City.

(2) The information derived from the portion of the card relating to "contacts" has given disappointing results. The work involved in following up and correlating cases amongst attendants on plague patients proved to be too great, since the attendants occasionally migrated to other quarters of the City where it was impossible to trace them. Moreover, the remarks we have just made on the question of the source of infection apply with equal force in this case.

Reference may be made to certain sources of error which are unavoidable in the collection of epidemiological data in an oriental city like Bombay. These may be summarised thus:—

(i) Cases which recover and which have not been attended by a medical practitioner are occasionally overlooked.

(ii) Undoubtedly in a number of instances plague deaths are falsely stated by the relatives to be due to other causes. The District Registrars are of course aware of this source of error and are able, in some instances at least, to make the necessary corrections.

(iii) Difficulties in the diagnosis of plague from other diseases, notably relapsing fever, must be considered.

We do not for a moment contend that errors due to these causes may not have crept into the information amassed on our behalf. We recognise, also, that the cases of plague recorded in detail on the cards are only a sample, although undoubtedly a very large one, of the total cases which actually occurred. Evidence however, which will be given later, shows that these errors are for practical purposes nullified by the large numbers of plague cases investigated (over 10,000). Further, we hope to show that the sample in almost all the districts was an extremely good one, so much so that it has amply fulfilled our main purpose, namely, the correlation of human and rat plague.

Besides the "human case" cards another method of studying the epidemic remains to be described. Maps of every section in Bombay were specially prepared for us. These are essentially street-maps, unnecessary details being avoided, and the streets and lanes being shown in plain black outline. Most of them are on a scale of 200 feet to the inch, the average size of the original maps being 24"  $\times$  18". Each District Registrar was supplied with copies of maps of the sections in his district. Every plague case, as it came to his notice, was represented by him on the map by a conventional sign together with the date when the case was reported to the Health Officer. The sign and date were allocated to a position on the map corresponding as nearly as possible to the house in which the patient was found. Distinctive signs were adopted to indicate a suspicious case of plague and a case which was imported either from outside Bombay or from another section. As a rule one map was used for the plague cases occurring during one month, but in some sections during the epidemic period it was found necessary in order to avoid overcrowding to use a fresh map every fortnight. The maps and case cards were checked by Drs Turkhud and Nerurkar and by members of the Commission.

Before bringing to a close this account of our methods of observing the epidemic in Bombay, we may refer to certain advantages which accrued from the system adopted, apart from the results which have emerged from an analysis of the case cards and maps.

(1) The scheme gave us abundant opportunities of observing with our own eyes the actual conditions in which the inhabitants of Bombay live, an experience which has assisted us greatly in coming to a conclusion as to the part played by these conditions in the spread of plague in the city.

(2) By the co-operation of the District Registrars we were enabled to obtain early information regarding incidents of special interest which occurred throughout the city, *e.g.* the occurrence of severe outbreaks in certain localities and buildings.

(3) It was possible to carry out special experiments in certain badly infected houses, which were brought to our notice by the District Registrars. Some of these have already been recounted in previous volumes of these reports, namely, those dealing with the infectivity of certain plague houses and the nature of the infecting agent within them.

## II. THE EPIZOOTIC.

The methods adopted for studying the epizootic may be described under three headings: (1) the collection of the rats, (2) the examination of the rats at the laboratory, and (3) the arrangements made to correlate the epizootic with the epidemic.

### 1. *The collection of the rats.*

Arrangements were made for a daily supply of several hundred rats (alive and dead) from all over the city. The rats were collected on our behalf by the Sanitary and Cleansing Department of the City.

In connection with the work of this Department there are eight Municipal stables or dépôts situated in the different districts. Each of these is in charge of an European Inspector, who is assisted by one or two sub-inspectors. The actual cleansing staff consists of 4800 "sweepers"—men and women. These persons are grouped into batches of 10 or 12, each of which is supervised by a "muccadam" or overseer, a man who is able to write the vernacular. The sweepers remove all refuse from the houses and gullies and sweep the streets. The gullies are cleaned by them at least once a day. Each man is allotted to a very small area so that the work may be efficiently done. The sweepers are the persons who are most likely to find dead rats, because if a rat is found dead in a house the occupants throw it out into the adjoining gully or into the street.

The sweeper when he found a dead rat took it to his muccadam, who attached to it a note stating the exact locality where the rat was found. The rat was then taken by the sweeper to the stable in his district, where roll call is held every morning, and he was paid  $\frac{1}{4}$  anna for every dead rat.

At the stable all the dead rats found at one address were put into a tin box, on the lid of which was a number.

The details concerning all the dead rats brought were entered into a form showing (1) the number of the box, (2) the number of rats in the box, (3) the address where the rats were found and (4) the name of the sweeper who found them. All the tins were then packed into carts and were sent with the corresponding form to the laboratory.

With regard to the capture of the live rats a number of traps were kept by the officials at each stable and these were distributed daily amongst selected sweepers (rat catchers), who set them in gullies, godowns, houses, stables, etc. Each trap was numbered and this number and other

particulars were entered on the form already described. The form was sent to the laboratory with the traps. A reward of  $\frac{1}{2}$  anna was given for each live rat.

## 2. *Examination of the rats at the laboratory.*

On arrival at the laboratory the rats were dealt with on the following scheme, which was so arranged that the rats passed through the hands of a series of unskilled native assistants, each of whom, however, was trained to his particular item of work and was competent to perform it.

The procedure differed slightly in the case of dead and live rats.

*Dead rats.* A card partly filled up by a clerk from the form sent from the stable was attached to the fore-leg of every dead rat. The details on this card are reproduced (II.). The rat was then dipped into an antiseptic solution in order to diminish the nuisance from flies in the subsequent operations. The next step was to weigh the rat on a spring balance and to enter the weight in grammes on the card. The rat was then pinned upon a small wooden board and taken to the man who dissected them. As will be seen from Plates XIX and XX the pinned-out rats were laid in rows on a series of long tables. A "cutter up" and a clerk were allotted to each table. The work of dissecting the rats was performed by three disciplined European soldiers on the staff of the Plague Research Laboratory, specially chosen for their intelligence and good conduct and trained for this particular duty by members of the Commission.

The "cutter up" opened the rat by an incision which included the groin, axilla and neck on both sides so as to expose the glands in these regions. He dictated to the clerk who accompanied him the species and sex of each rat and the presence or absence of pregnancy in the females. These facts were noted on the rat cards by the clerk.

The methods pursued in the diagnosis of plague-infected rats have been already fully described<sup>1</sup>, so that it is unnecessary to do more than briefly refer to them. Diagnosis of plague rats by naked-eye examination was chiefly relied upon, since it has been our experience that in the hands of competent observers it is the best single method of diagnosis. Every rat dissected throughout the entire investigation, without exception, whether infected or apparently healthy, was examined by a member of the Commission, who decided as to the diagnosis. Microscopical

<sup>1</sup> Vol. VII. p. 339.





Bombay City: rat examination at the Laboratory.







Bombay City: rat examination at the Laboratory.



Form II.

Date \_\_\_\_\_ Number \_\_\_\_\_

Catcher \_\_\_\_\_

Locality \_\_\_\_\_

Where & how caught \_\_\_\_\_

Weight \_\_\_\_\_ Number of fleas \_\_\_\_\_

Species \_\_\_\_\_

Sex \_\_\_\_\_ Pregnant \_\_\_\_\_

Infected \_\_\_\_\_

Initials.

Form III.

Date \_\_\_\_\_ District \_\_\_\_\_ Section \_\_\_\_\_

Catcher \_\_\_\_\_

Address \_\_\_\_\_

No. of rats in cage \_\_\_\_\_ Species \_\_\_\_\_

{ Total No. of fleas \_\_\_\_\_  
Average No.  
for rat \_\_\_\_\_

Gully

House

Stable

Godown

Remarks :—

examinations of organ-smears and animal tests were used only when the diagnosis was uncertain.

*Live rats.* The procedure adopted in the case of the rats caught alive in traps was as follows. The rats first of all were cleared of their fleas (Plate XXI). At an early period of the investigation an attempt was made to obtain a daily average flea-count, but as the arrangements were somewhat defective in several respects we need only describe an improved method (Plate XXII) which was commenced in October 1906. The arrangements at this later period were made so as to fulfil two purposes: (1) the exact allocation of each trap containing rats to a particular part of an inhabited building, *e.g.* the ground floor, first floor, etc. or to a godown, stable or gully, and (2) an average flea-count, not only for the total rats but for each species of rat and for the rats found in particular classes of buildings, *e.g.* houses, godowns, etc. This scheme was entrusted to specially selected men at the municipal stables and on the District Registrars' staff who distributed traps to selected sweepers.

The traps were purposely placed in various situations and when a "take" was made, the man in charge wrote on the back of a specially designed card (III.) the exact situation where the trap was found. The trap was then taken to the stable or to the District Registrar's office, where this information, if in the vernacular, was translated and written on the printed side of the card. The traps were afterwards sent to the laboratory. In this scheme we adopted the device of enclosing the whole trap, immediately after it was found to contain rats, in a stout canvas bag made for the purpose and bearing a number. The card referred to above was attached to the neck of this bag.

The object of the bag was in order to secure more uniform results in the flea-counts. It would appear that in the journey to the laboratory fleas on rats in open wire traps exposed to the sunlight dropped off in considerable numbers. By comparison with the old method it was proved that more uniform flea-counts were obtained by the use of this device and that the average number of fleas found per rat was higher.

When the traps arrived at the laboratory each trap was removed from the bag and both trap and bag were at once put into a tin box, to which chloroform was added. Four of these boxes were in constant use during the flea examination. Each of them had a metal tray resting on the bottom, on which tray the trap and bag were placed. The tray and its contents were removed *en bloc* after the rats were killed by the chloroform. The fleas on the tray were then counted and those on each rat separately, a note of the results being made on the card and on a form.





Bombay City: catching fleas.





Bombay City: catching fleas, note the traps enclosed in canvas bags.



Register No. Serial No. See also No.	Date of examination	Locality where caught	Catcher's name	Weight	Dead or alive	Species	Sex	Pregnant
XIII								
<b>Diagnosis</b>  From p.m. appearances "   microscopical examination "   detailed examination Confirmed by animal test				District and Reporter's name				
				Exact locality where rat found				
				Information from inhabitants as to rat mortality in neighbourhood				
				Date of attack of last nearest human case				
				Reference to later associated human cases				
				Reference to associated plague rats				
<i>Post-mortem appearances</i>  <i>Naked eye.</i> Condition ;   Putrid,                      emaciated, Rigor mortis Primary bubo Secondary glands Subcutaneous congestion Liver Spleen Intestines Kidneys Lungs Pleural effusion Haemorrhages <i>Microscop.</i> Heart blood Spleen Bubo  <i>Cultures</i> <i>Animal tests</i>								



Remarks

Description of building nearest locality where infected rat was found

Reporter's name	District	Date of visit
<i>Adjoining block of houses.</i>		
No. of houses in	Address	
No. of storeys		
No. of inhabitants		
Shops on ground floor		
If rat found in gully condition of gully		
General sanitary condition of house		
Circumstances in house favouring rats		
<i>Adjoining Godown</i>		
No. of storeys	Address	
Floor		
Ceiling		
Tiles		
Light		
Ventilation		
Kind of business carried on		
Direct evidence as to occurrence of rats		
Circumstances in building favouring rats		
Proximity of building to grain-godown etc.		
" " " gully		
Condition of adjoining gullies		

A complete record of the information obtained in this way was kept in a ledger.

The subsequent treatment of the trapped rats was the same as has been described in the case of the dead rats.

It remains to be said that the whole of the information noted on the rat cards was daily recorded in ledgers. In addition daily, weekly and monthly summaries of the rat and flea statistics were prepared.

3. *The arrangements for correlating the epizootic with the epidemic.*

A list of plague-infected rats for each day was sent as soon as possible to each District Registrar and to the Inspectors at each stable. On the same evening or next morning the sweepers who picked up the plague rats pointed out the exact locality where they had found them to the mucedums, who marked the place with a sign "P. R." and the date in red paint (see Plate XXVI.).

The District Registrar or his sub-registrar on visiting the place later in the day recognised it from this sign and filled up, on the spot, a specially designed "plague rat" card which had been sent him from the laboratory already partially filled up, *i.e.* giving the species of the rat, address where it was found and the name of the catcher. The Registrar at the same time allocated the plague rat to its proper position on the map of the section which we described in the account of the epidemic.

A distinctive sign was used for each species, *Mus rattus* and *Mus decumanus*, and the date when the rat was sent to the laboratory was placed alongside. After being filled up the cards were returned to the laboratory where they were copied by clerks into duplicate cards (XIII.) with additional headings (already entered) relating to details of the post-mortem examination and diagnosis of the rat. The maps and cards were checked by members of the Commission, assisted by Drs Turkhud and Nerurkar.

Although the methods for allocating the plague-infected rats were apparently the best that could be used under the circumstances, yet we cannot pretend that absolute accuracy was secured in placing every rat. We have good reason to believe, however, that the accuracy of the methods although only approximate was sufficient for the purpose of comparing the plague rats with the human cases in each section and even over smaller areas than sections.

## IV. THE EPIZOOTIC.

## I. AN ACCOUNT OF THE RODENTS MET WITH IN BOMBAY.

Before entering upon a description of the epizootic it is necessary to give a brief account of the rodents which have been met with in the course of our work in Bombay.

(1) *The species of rodents found infected with plague in nature.*

These are: *Mus rattus* (the house rat), *Mus decumanus* (the so-called brown, gray or sewer rat), *Mus musculus* (mouse), *Nesokia bengalensis* and *Nesokia bandicota* (bandicoot).

On account of the large numbers of rodents brought daily to the laboratory for examination it was necessary to come to a conclusion regarding their species from external appearances alone. The mouse and the bandicoot offered no difficulty in identification. With regard to *Mus rattus* and *Mus decumanus*, the most important of all in relation to the epidemic, it was soon found that with practice no difficulty was experienced in differentiating them. We have satisfied ourselves that it is always possible to distinguish rats of the type of *Mus rattus* from rats of the type of *Mus decumanus*. Since rats of an intermediate type have never been found by us it would appear that the species do not interbreed.

*Nesokia bengalensis* on superficial examination resembles *Mus decumanus* so closely that its occurrence amongst the rats was overlooked during the earlier period of the investigation. Nevertheless after the recognition of this rodent as a distinct species we were able with a little practice readily to identify it from its external appearances alone. It may be added that for reasons which will appear later the error introduced into our work by the confusion of *Nesokia bengalensis* with *Mus decumanus* may be said to be negligible.

In the following description of *Mus rattus*, *Mus decumanus* and *Nesokia bengalensis* we shall confine ourselves to the points of distinction on which we relied throughout our work.

*Mus rattus*.—Tail longer than the body and head together; dark in colour compared with the tail of *Mus decumanus* and uniform in colour all round. The scales on the tail are arranged in rings; these rings are better marked than in *Mus decumanus*. The ears are larger in proportion to the size of the rat than in *Mus decumanus*. The colour of the fur, especially on the ventral aspect of the body, is very variable. *Mus rattus* is frequently called the black rat, but the commonest

type in Bombay is a brown variety. The black variety is somewhat rarely seen in rats taken from the City, though it occurs more commonly amongst rats trapped from ships in Bombay harbour. The belly is invariably of a lighter colour than the back. Rarely the belly is unusually light coloured; rats with this peculiarity are considered by some workers as a variety (*Mus alexandrinus*), but no distinction was made by us on this account. We have met with pure and partial albinos but they are very rare. *Spines* in the fur of the back are common.

*Mus decumanus*.—*Tail shorter than body and head together*. The ventral aspect of the tail is lighter coloured than the dorsal aspect. The ears are smaller in proportion to the size of the rat than in *Mus rattus*. The colour of the fur is much more constant than in *Mus rattus*. It is brownish-gray on the back and a lighter gray on the belly. Pure and partial albinos have been rarely met with.

*Nesokia bengalensis*<sup>1</sup>.—*Tail shorter than head and body*, in this respect resembling that of *Mus decumanus*, but in appearance resembling that of *Mus rattus*, i.e. the rings are well marked. The hairs on the tail are shorter and less numerous than in *Mus decumanus* and there is no brush of hairs projecting beyond the tip of the tail as there is in *Mus decumanus*. The head when looked at from above is broader and shorter than in *Mus decumanus*. The ears are somewhat larger in proportion to the size of the animal than in *Mus decumanus*. The fur is coarse with well-marked spines on the back. The colour of the fur is uniformly darker than in *Mus decumanus* and this colour is very constant. *Nesokia* grunts when alarmed, whereas *Mus decumanus* squeals. There is a well-marked bony tubercle on the external surface of the ramus of the lower jaw contrasting with a similar but rudimentary tubercle in the case of *Mus decumanus*.

Musk rats were trapped in considerable numbers from all parts of the Island. This animal (*Crocidura coerulea*: order—Insectivora) is not a rat but a shrew. It is recognised with great ease chiefly by its long head and pointed snout.

## (2) General distribution and abundance of each species in the rodent population.

In Bombay city *Mus rattus* and *Mus decumanus* both occur in prodigious numbers. In the country villages, however, *Mus decumanus* is very rarely found (see Table III.). This is doubtless correlated with the absence of any sewerage system in those villages.

There can be no doubt that the species of rodents which are most numerous in Bombay are *Mus rattus* and *Mus decumanus*. It is difficult from the figures given in Tables I. and II. to arrive at a definite conclusion as to whether *Mus rattus* or *Mus decumanus* is the predominating

<sup>1</sup> "It is doubtful whether this (genus *Nesokia*) should rank as more than a subgenus of *Mus*." *The Fauna of British India: Mammalia*. W. T. Blanford, F.R.S., London, 1888—1891, p. 422.

species, since the "takes" depend to a great extent on the number of traps set in situations frequented by each species. More than twice as many *Mus rattus* were trapped alive as *decumanus*, while the proportion is reversed in the case of rats found dead. This fact may be explained by the habits of each species, which are such that dead *decumanus* would be much more likely found than dead *rattus*.

However this may be, the point is of little practical importance, since it is certain that enormous numbers of each species exist in Bombay and that both species are very liable to plague infection.

The number of mice brought for examination during the year was small, probably for the reason that the rat traps used were not well suited for trapping mice. From our observations in the City, however, we are inclined to think that the mouse population is a small one, relatively at least to that of *Mus rattus* and *Mus decumanus*, and that

TABLE I.

*Showing the result of rat trapping in Bombay for three months,  
November—January.*

	Takes	Rats	<i>rattus</i>		<i>decumanus</i>		<i>Nesokia</i>		Musk rats		Mice	
			Total No.	% to Total	Total No.	% to Total	Total No.	% to Total	Total No.	% to Total	Total No.	% to Total
Total Number	2901	12856	8332	66.2	3618	28.7	123	1.0	294	2.3	219	1.7
Gullies	278	1254	701	55.9	485	38.7	22	1.7	26	2.0	20	1.6
House—	2253	9247	6466	69.9	2386	25.8	75	0.8	181	2.0	139	1.5
Compound	16	104	33	31.7	62	59.6	—	—	5	4.8	4	3.8
Ground floor	1384	5855	3805	65.0	1774	30.0	69	1.2	132	2.3	75	1.2
1st „	479	1971	1536	77.9	390	19.8	3	0.1	24	1.2	18	0.9
2nd „	234	845	720	85.2	105	12.4	—	—	10	1.2	10	1.2
3rd „	80	260	224	86.2	36	13.8	—	—	—	—	—	—
4th „	15	71	43	60.6	—	—	—	—	2	2.8	26	36.6
5th „	—	—	—	—	—	—	—	—	—	—	—	—
6th „	1	1	—	—	—	—	—	—	1	100.0	—	—
Top „	16	54	48	88.9	—	—	—	—	—	—	6	11.1
Not stated	28	86	57	66.3	19	22.0	3	3.5	7	8.1	—	—
Stables—	170	944	484	51.3	352	37.3	18	1.9	54	5.7	36	3.8
Horse	118	669	321	47.9	276	41.3	1	0.1	38	5.7	33	4.9
Bullock	16	59	25	42.3	10	16.9	17	28.8	7	11.8	—	—
Not stated	36	216	138	63.9	66	30.5	—	—	9	4.2	3	1.4
Godowns—	136	846	540	63.8	268	31.7	3	0.4	16	1.9	19	2.2
Food	32	305	228	74.7	63	20.7	—	—	6	2.0	8	2.6
Not food	97	517	290	56.0	203	39.2	3	0.6	10	1.9	11	2.1
Gunny Bags	1	3	3	100.0	—	—	—	—	—	—	—	—
Not stated	6	21	19	90.5	2	9.5	—	—	—	—	—	—
Food, Teashops	50	247	108	43.7	117	47.4	1	0.4	16	6.4	5	2.0
Unclassified	14	48	33	68.7	10	20.8	4	8.2	1	2.0	—	—



TABLE II.

Showing relative distribution of *M. rattus* and *M. decumanus* in Bombay—trapping for three months, November—January.

	Rats	<i>Rattus</i>		<i>Decumanus</i>	
		Total No.	% to total rats	Total No.	% to total rats
Total Number	11950	8332	69·7	3618	30·3
Gullies	1186	701	59·1	485	40·9
House	8852	6466	73·0	2386	27·0
Compound	95	33	34·7	62	65·3
Ground floor	5579	3805	68·2	1774	31·8
1st „	1926	1536	79·7	390	20·3
2nd „	825	720	87·3	105	12·7
3rd „	260	224	86·2	36	13·8
4th „	43	43	100·0	—	—
5th „	—	—	—	—	—
6th „	—	—	—	—	—
Top „	48	48	100·0	—	—
Not stated	76	57	75·0	19	25·0
Stables—	836	484	57·9	352	42·1
Horse	597	321	53·8	276	46·2
Bullock	35	25	71·4	10	28·6
Not stated	204	138	67·6	66	32·4
Godowns—	808	540	66·8	268	33·2
Food	291	228	78·3	63	21·6
Not food	493	290	58·8	203	41·2
Gunny Bags	3	3	100·0	—	—
Not stated	21	19	90·5	2	9·5
Food, Tea shops	225	108	48·0	117	52·0
Unclassified	43	33	76·7	10	23·3

TABLE III.

Showing number and species of rodents trapped alive in four villages in Bombay island.

Village	No. of <i>M. rattus</i> trapped	<i>M. decumanus</i> or <i>Nesokia bengalensis</i> trapped	Mice trapped	Musk rats trapped	Period of trapping	Human Population	No. of buildings in village
Parel	1762	2 <i>M. decumanus</i> 3 <i>Nesokia beng.</i> 1?¹	91	182	20. xi. 05—15. vii. 06	3718	150
Worli	2127	2 ?¹	8	592	22. xi. 05—15. vii. 06	2500	440
Wadhala	1120	3 <i>Nesokia bengalensis</i>	6	173	23. xi. 05—15. vii. 06	1922	192
Sion	528	1 ?¹	2	96	4. xii. 05—15. vii. 06	950	102
Total	5537	12	107	1043	20. xi. 05—15. vii. 06	9090	884

¹ Probably *Nesokia bengalensis*.

this is probably dependent upon the presence of *Mus rattus* in the houses.

*Nesokia bengalensis* is not, so far as we can ascertain, a common rodent in Bombay, at least near human habitations. In the City it chiefly occurs in those sections containing large areas of waste ground, and on the whole its haunts appear to be similar to those of *Mus decumanus* (see Table I.). It will be seen from Table I. that 1% of a total of 12,856 animals trapped alive belonged to this species and that only 123 *Nesokia bengalensis* were trapped as compared to 3618 *Mus decumanus*, i.e., they accounted for only 3·2% of the *decumanus*-like rodents.

Specimens of the bandicoot were brought to the laboratory very seldom. It is mostly found in open country and palm groves in the northern part of the Island. The musk rat is fairly common in Bombay.

### (3) *Remarks on the habits of each species.*

*Mus rattus* in Bombay is essentially a house rat. It is so confiding that it may almost be said to be a domesticated animal. The people regard its presence in their houses with the utmost tolerance, so that it takes up its abode, and even breeds, in their living rooms, amongst the little disturbed accumulations of rubbish so commonly found in native houses. Certain natives not only take no steps to rid their houses of rats but actually secure them from molestation. One sect, for example, the Jains, to whom every form of animal life is sacred, look upon rat destruction, even when adopted as a measure of plague prophylaxis, as an outrage against their religion, and refuse permission to have traps and baits placed in their houses.

*Mus rattus*, although typically a climbing rat, is able to burrow, e.g., in beaten earth floors. We have frequently made this observation and in one instance (in Parel village) have seen exceptionally large and numerous holes and burrows in the earthen floor of a store-room for grain from which many *Mus rattus* had been trapped. We may note that *Mus rattus* appears to be more particular in its choice of food than *Mus decumanus*. We have found that, when compared with the proportions in which rats are obtained from all over the City, the relative number of *Mus rattus* trapped increases in food-godowns (chiefly grain and seed godowns) and diminishes in non-food-godowns. The proportions are seen in the following table :—

	<i>M. rattus</i>		<i>M. decumanus</i>
Total number of rats trapped in various situations	230	to	100
"      "      "      "      food-godowns	371	to	100
"      "      "      "      non-food-godowns	141	to	100

It is probable that *Mus rattus* is largely a grain-eating rat.

Whereas the nests of *Mus decumanus* are almost invariably found in the burrows of this animal, the nests of *Mus rattus* are chiefly in little disturbed accumulations of material, such as stacks of firewood, cotton waste, etc. or in recesses, such as cupboards.

*Mus decumanus*, as is well known, is a rat which lives for the most part outside houses in sewers, storm-water drains, stables, etc. It is a burrowing animal with remarkable powers for gnawing through hard materials, *e.g.*, brick and concrete, but it is also a good climber. *Mus decumanus*, since it lives chiefly in the open, probably makes wider excursions in search of food than *Mus rattus*.

We have, however, observed nothing to show that these rats are in the habit of migrating, *e.g.*, from one quarter of the City to another. Such a migration would be determined chiefly by a lack of food supply, and it is certain that in Bombay the food supply of *Mus decumanus* is abundant everywhere at all periods of the year.

The following statements are founded on observations recorded in Table II., in which the results of trapping nearly 12,000 rats of these two species in the native City are summarised.

*Mus rattus* is apparently much more common in Bombay than *Mus decumanus*, as we caught, taking all traps set, seven of the former to every three of the latter.

They are found in this proportion in ground floors of houses, but *Mus rattus* increases relatively to *Mus decumanus* as one ascends the building. *Mus decumanus* has never been found above the third floor, so that on the fourth floor and upwards *Mus rattus* alone is found.

In gullies, compounds of houses, stables, non-food-godowns, and food and tea-shops the number of *Mus decumanus* relatively increases, so much so in the case of compounds, namely, gardens and open spaces around houses, that we caught in traps set there about twice as many of this species as of *Mus rattus*.

Further, it is evident that these two species of rats are closely associated with each other, gullies, the lower floors of houses and godowns appearing to be their common meeting ground.

It is necessary to emphasise two important facts concerning the rats of Bombay. The first is the widespread distribution of *Mus rattus*

in buildings in the City. We do not think it an exaggeration to state that every inhabited building in Bombay City and Island, not excepting even the better class bungalows, shelters its colony of *Mus rattus*. The second important fact is, that to a certain extent *Mus decumanus* in Bombay is a house rat. We have actually seen extensive burrows of *Mus decumanus* opened up in the "chunam" floor of the living room of a house on the first floor (*i.e.* above the ground floor) of a typical chawl. Several adults and a large number of newly born rats were captured in the burrows. On another occasion we obtained two plague-infected *Mus decumanus* from the second floor of an office in the Fort section.

TABLE IV.

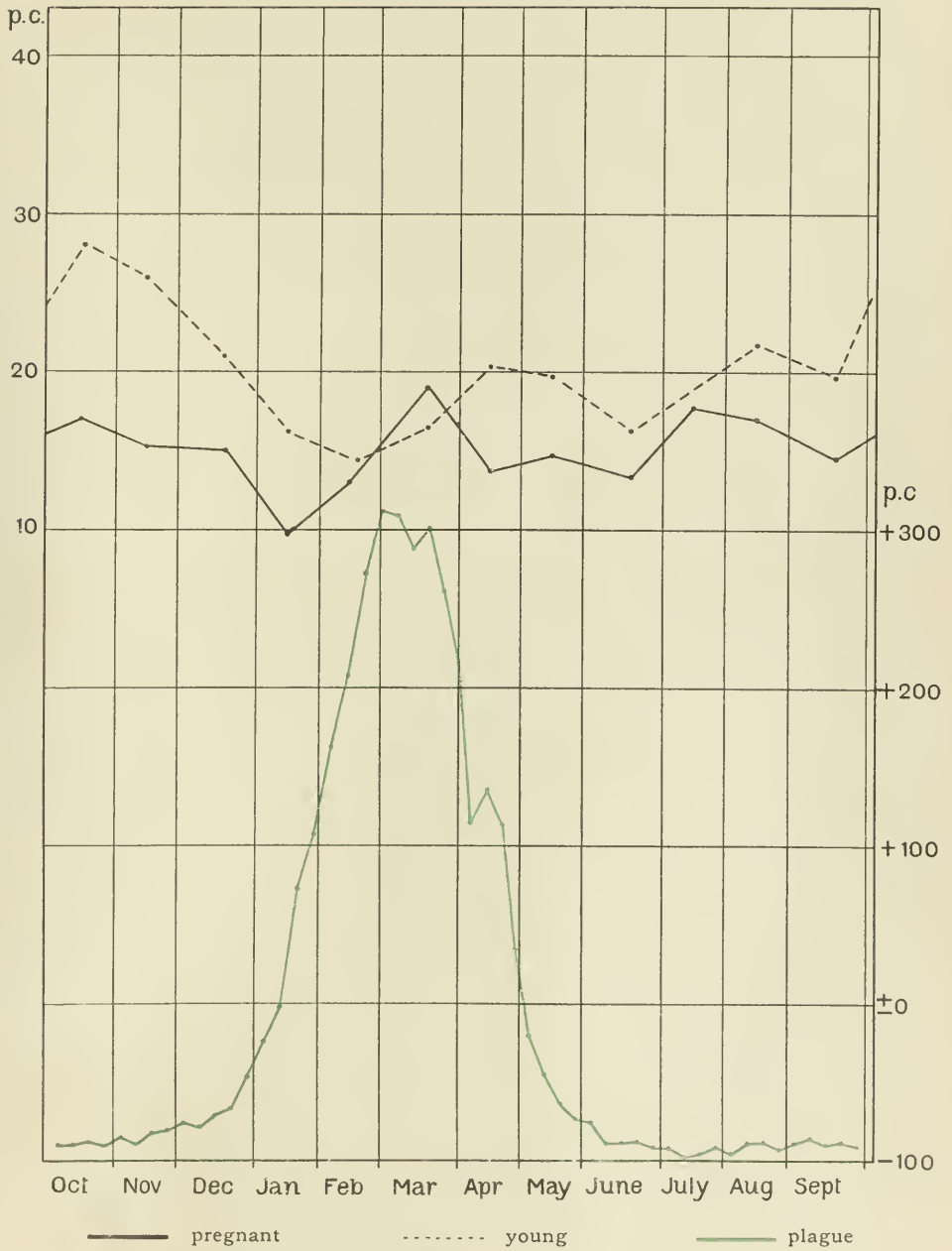
*Showing the breeding season of M. decumanus.*

Month	Total rats examined			Total young rats			Percentage of young rats on total rats		
	Live	Dead	Total	Live	Dead	Total	Live	Dead	Total
October	1702	2250	3952	712	395	1107	41.8	17.5	28.0
November	1294	2419	3713	546	417	963	42.2	17.2	25.9
December	1824	3497	5321	562	553	1115	30.2	15.8	21.0
January	1675	11282	12957	459	1645	2104	27.4	14.6	16.2
February	706	12790	13496	196	1738	1934	27.7	13.6	14.3
March	520	9959	10479	170	1547	1717	32.7	15.5	16.4
April	536	6124	6660	182	1159	1340	33.9	18.9	20.1
May	486	3635	4124	149	668	817	30.4	18.4	19.8
June	474	1802	2276	168	198	366	35.3	11.0	16.1
July	669	1818	2487	221	247	468	33.0	13.5	18.8
August	665	1820	2485	270	275	545	40.6	15.1	21.9
September	630	2209	2839	204	360	564	32.4	16.3	19.9
							34.0	15.6	19.9

	Total adult females			Total found pregnant			Percentage pregnant		
	Live	Dead	Total	Live	Dead	Total	Live	Dead	Total
October	559	836	1395	125	112	237	22.3	13.4	17.0
November	395	872	1267	66	128	194	16.7	14.7	15.3
December	641	1243	1884	102	184	286	15.9	14.8	15.2
January	584	4534	5118	84	417	501	14.3	9.2	9.8
February	276	4978	5254	48	634	682	17.4	12.7	13.0
March	188	3662	3850	35	689	724	18.6	18.8	18.8
April	205	2264	2869	48	351	399	23.4	15.5	13.9
May	210	1513	1723	47	210	257	22.4	13.9	14.9
June	188	758	946	39	96	135	20.7	12.7	14.3
July	254	764	1018	54	127	181	21.2	16.6	17.8
August	229	702	931	47	111	158	20.5	15.8	17.0
September	225	798	1023	47	100	147	20.9	12.5	14.4
							19.5	14.2	15.1

# BOMBAY CITY I



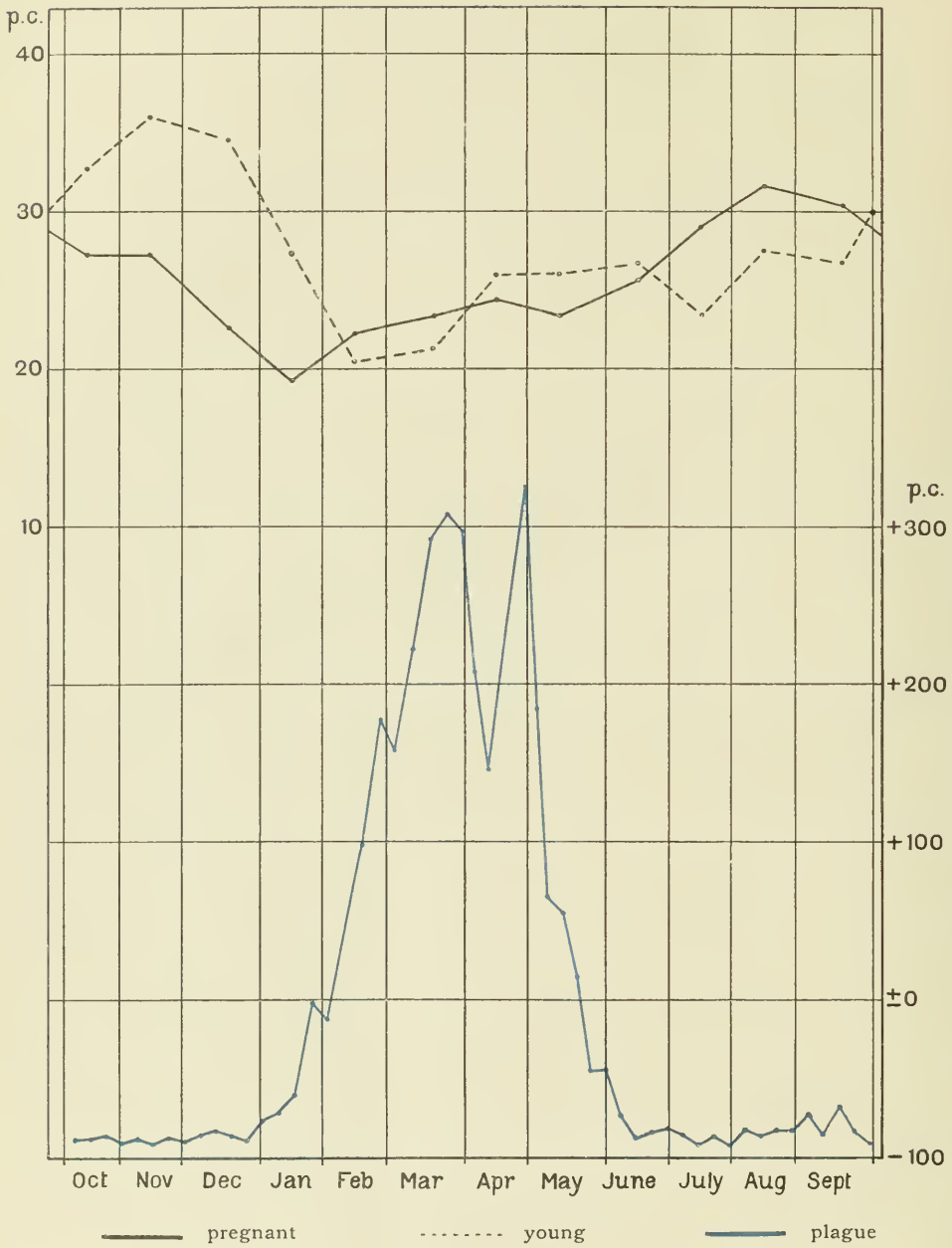
Breeding and plague in *Mus decumanus*







# BOMBAY CITY II



Breeding and plague in *Mus rattus*

(4) *The breeding season of Mus rattus and Mus decumanus.*

It was sought to investigate this question by considering (1) the percentage of adult females found pregnant, and (2) the proportion of young rats found, at different seasons. For this purpose "young" *rattus* and *decumanus* are arbitrarily defined as those weighing 70 and 100 grammes or less respectively; no rats below these weights were found pregnant. The results are shown in Tables IV. and V. and Charts I. and II.

The curves for pregnant females and for young rats correspond fairly well, and indicate that, though in Bombay there is no very definite

TABLE V.  
*Showing the breeding season of M. rattus.*

Month	Total rats examined			Young rats			Percentage of young rats on all rats		
	Live	Dead	Total	Live	Dead	Total	Live	Dead	Total
October	2101	1071	3172	739	305	1044	35.1	28.5	32.9
November	1678	1100	2778	659	342	1001	39.2	31.0	36.0
December	3268	1632	4900	1291	392	1683	39.5	24.0	34.4
January	2833	3087	5920	978	637	1615	34.5	16.5	27.3
February	1427	3864	5291	418	668	1086	29.3	18.3	20.5
March	811	4127	4938	293	757	1050	36.1	18.3	21.3
April	1104	3445	4549	413	770	1183	37.4	22.3	26.0
May	900	2093	2993	354	423	777	39.3	20.2	26.0
June	1011	1135	2146	375	196	571	37.0	17.3	26.6
July	1693	1292	2985	489	210	699	28.8	17.0	23.4
August	1864	1409	3273	648	251	899	38.7	17.8	27.5
September	1801	1536	3337	598	298	896	33.2	19.4	26.8
							35.7	20.9	27.4

	Total adult females			Total found pregnant			Percentage pregnant		
	Live	Dead	Total	Live	Dead	Total	Live	Dead	Total
October	761	364	1125	224	83	307	29.3	22.8	27.3
November	574	325	899	145	100	245	25.2	30.8	27.2
December	1056	599	1655	262	114	376	24.7	19.0	22.7
January	961	1165	2126	210	201	411	21.6	17.3	19.3
February	562	1360	1922	149	280	429	22.9	20.6	22.3
March	277	1462	1739	81	350	431	29.2	23.9	24.8
April	394	1244	1638	108	296	404	27.4	23.8	24.7
May	282	968	1250	95	203	298	33.7	21.0	23.8
June	360	460	820	117	95	212	32.5	20.7	25.9
July	679	464	1143	225	107	332	33.1	23.0	29.0
August	713	534	1237	238	154	392	33.3	29.4	31.7
September	682	581	1263	246	134	380	36.0	23.0	30.1
							29.1	22.9	25.6

breeding season, both species breed most freely in the hottest part of the year (June to October). It will be noted that the fall in fertility begins before the onset of the plague epizootic, though it roughly coincides with it later on.

The average number of foetuses in 114 *Mus rattus* was 5·2, the largest number found being 9; for 71 *Mus decumanus* the figures were 8·1 and 14.

(5) *Relative incidence of plague in Mus rattus and Mus decumanus.*

The percentage of *Mus rattus* found infected with plague was considerably smaller than that of *Mus decumanus*.

TABLE VI.

*Showing the age and sex incidence of plague among rats.*

	<i>M. decumanus</i>			<i>M. rattus</i>		
	Examined	Plague infected	Per cent.	Examined	Plague infected	Per cent.
<i>Live rats.</i>						
Old males	3391	45	1·3	5945	34	0·6
Old females	3955	32	0·8	7296	23	0·3
Young males	1814	8	0·5	3545	6	0·2
Young females	2024	10	0·5	3705	12	0·3
Old rats	7346	77	1·0	13241	57	0·4
Young rats	3838	18	0·5	7250	18	0·2
Male rats	5205	53	1·0	9490	40	0·4
Female rats	5979	42	0·7	11001	35	0·3
Total	11184	95	0·85	20491	75	0·37
<i>Dead rats.</i>						
Old males	27414	6691	24·3	11299	2183	20·3
Old females	22961	4377	19·1	9434	1451	15·7
Young males	4303	964	22·4	2520	319	12·7
Young females	4927	1150	23·3	2558	353	13·3
Old rats	50375	11068	22·0	20733	3634	17·6
Young rats	9230	2114	22·9	5078	672	13·3
Male rats	31717	7655	24·2	13819	2502	18·1
Female rats	27888	5527	19·9	11992	1804	15·0
Total	59605	13182	22·2	25811	4306	16·7
<i>All rats.</i>						
Old males	30805	6736	21·8	17244	2217	12·9
Old females	26916	4409	16·4	16730	1474	8·8
Young males	6117	972	15·9	6065	325	4·9
Young females	6951	1160	16·7	6363	365	5·7
Old rats	57721	11145	19·3	33974	3691	10·8
Young rats	13068	2132	16·3	12328	690	5·7
Male rats	36922	7708	20·9	23309	2542	10·9
Female rats	33867	5569	16·5	22993	1839	8·0
Total	70789	13277	18·8	46302	4381	9·45



The monthly figures are as follows and show that this difference obtains both during the epizootic and in the off-plague season.

TABLE VII.

1905	October	1.9	0.8
	November	4.3	0.7
	December	6.3	1.0
1906	January	14.4	4.6
	February	25.7	14.6
	March	39.8	28.0
	April	31.4	25.2
	May	14.2	15.6
	June	5.3	3.3
	July	3.9	1.6
	August	5.4	2.3
	September	4.6	2.3
	Average	13.1	8.3

The experimental susceptibility of the two species is about the same. We have many reasons for believing that rats in nature are infected by the agency of fleas. We therefore compared their susceptibilities by adding infected fleas to rats in flea-proof cages, thereby imitating as nearly as possible their natural mode of infection. In every experiment approximately the same number of fleas were used. Some of the experiments were carried out during the epizootic season of 1906, while the remainder were carried out in the epizootic season of 1907. In all 100 experiments have been done with the following results:

33 experiments with <i>Mus decumanus</i>	16 successes = 48 %
67     ,,     ,, <i>Mus rattus</i>	29     ,,     = 43 %
Total	45     ,,     = 45 %

These figures make it clear that the two species of rats show practically no difference in their susceptibility to plague by flea transmission.

It would appear, therefore, that the greater incidence of plague amongst *Mus decumanus* as compared with *Mus rattus* cannot be explained on the ground of a difference in susceptibility.

It follows that the explanation must lie in the opportunities for infection being greater in *decumanus* than in *rattus*. We have in fact found that the flea infestation of *decumanus* is much greater, as the following abstract from a complete series of counts to be dealt with later shows.

TABLE VIII.

		<i>M. decumanus</i>		<i>M. rattus</i>	
		Rats examined	Fleas per rat	Rats examined	Fleas per rat
1906	November	412	5·7	1313	2·5
	December	489	5·7	2087	2·6
1907	January	465	9·0	1927	3·2
	February	309	11·9	1693	4·5
	March	300	12·8	1799	5·2
	April	306	13·9	1911	5·2

We could not find from our figures that plague was specially incident upon male or female, old or young rats of either species.

## II. THE RELATION OF THE EPIZOOTIC AMONGST THE RATS TO TIME AND PLACE.

It is necessary at the outset to insist upon the fact that in Bombay City there is a *Mus decumanus* epizootic and a *Mus rattus* epizootic. That they must be considered as separate epizootics will appear shortly, when we discuss the time and place relations of each. We may add that, although distinct, the epizootics cannot be dissociated, the one having a definite relation to the other. It will be convenient therefore to discuss them together.

### THE EPIZOOTICS IN RELATION TO TIME.

#### (a) *Their seasonal prevalence.*

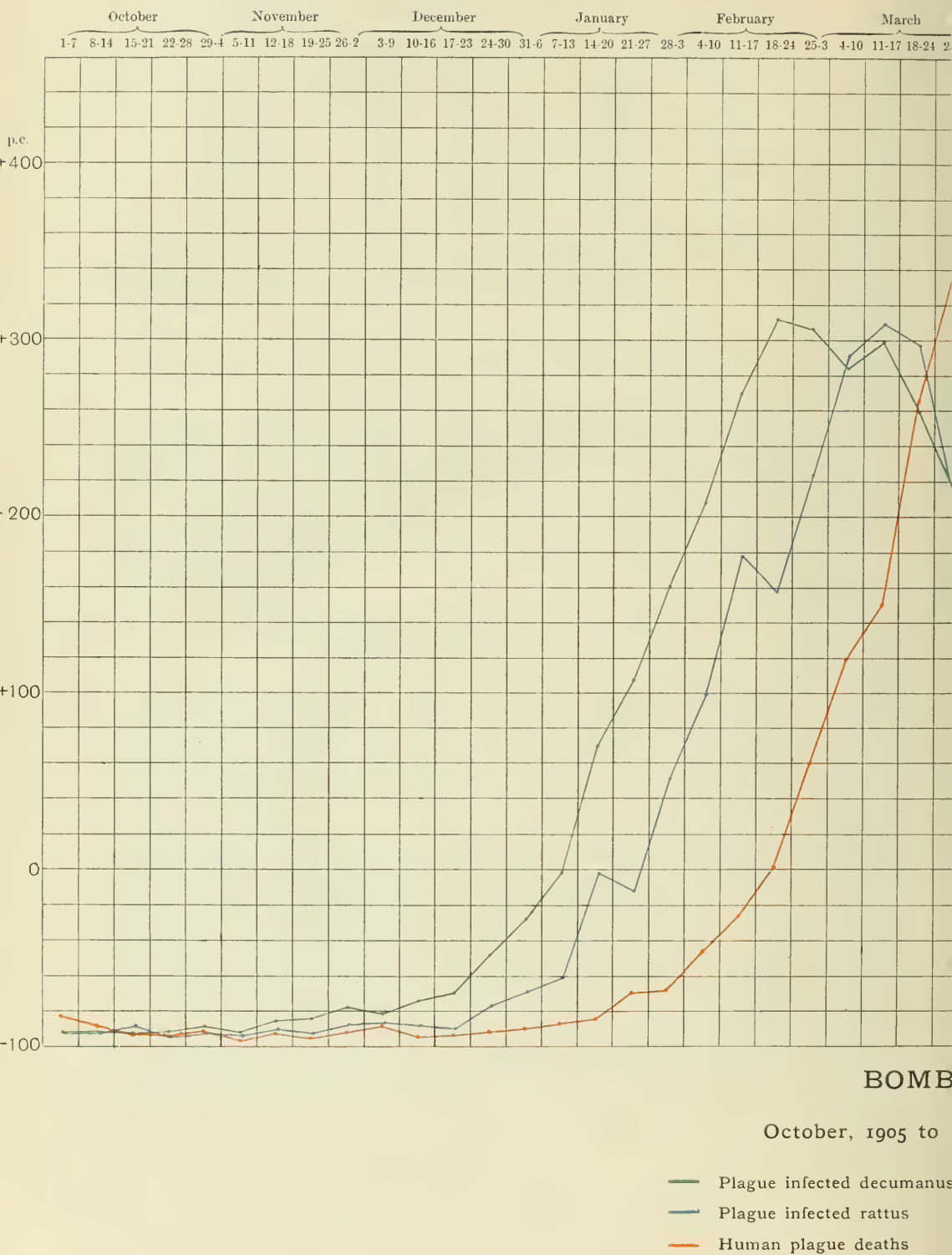
It will be seen from the charts<sup>1</sup> of Bombay City (Chart III.), and of certain of the sections (Charts IV.—XV.) which display the relation between rat and human plague, that the epizootics present well defined seasonal variations. Since, as will also be readily observed, the epizootics

<sup>1</sup> These charts have been drawn up in the following way. In that for Bombay City the data for most of the sections in the island have been collated. The data of certain sections in the north of the island have been omitted on account of a defective rat collection due to the large area and sparse population of these sections. The weekly figures of plague-infected rats of each species for the remaining sections were corrected for a week of seven days, in order to eliminate variations due to differences in the number of days per week the rat investigation was carried out. The corrected figures for the selected sections for weekly periods having been added together a mean of the resulting figures for the year was taken, and each of the weekly figures was then expressed as a percentage in terms of the mean of the year. The chart was constructed from these percentages. The charts for the sections differ from that of the City only in that the periods are fortnightly instead of weekly. The crude figures actually obtained are shown in Table XXIV. p. 796. There is very little difference between these and the corrected data.

# CHART III

## BOMBAY

October, 1905 to September, 1906

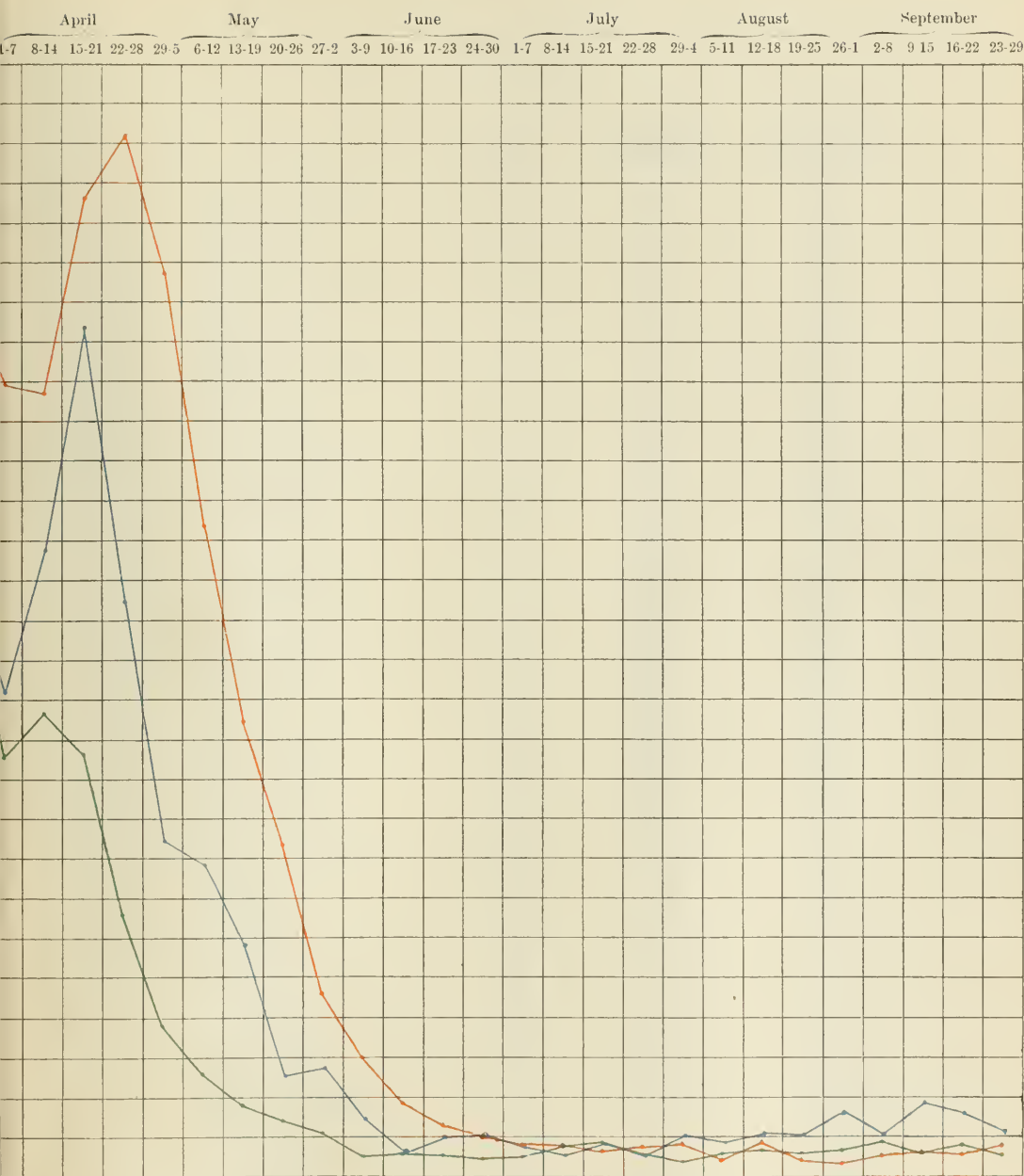


BOMB

October, 1905 to

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths

CHART III



September, 1906

Expressed as a p.c. above  
and below the mean





TABLE IX.

<i>Bombay.</i>			
Weekly periods	Plague mortality	Plague infected <i>Rattus</i> corrected for week of 7 days	Plague infected <i>Decumanus</i> corrected for week of 7 days
1st to 7th October	31	5·9	16·4
8th to 14th "	18	5·9	19·7
15th to 21st "	12	8·2	23·4
22nd to 28th "	9	3·5	18·6
29th to 4th November	13	5·9	30·3
5th to 11th "	6	2·8	21·0
12th to 18th "	9	6·9	42·0
19th to 25th "	5	5·9	47·9
26th to 2nd December	9	9·4	67·7
3rd to 9th "	16	11·7	60·5
10th to 16th "	8	9·5	78·1
17th to 23rd "	8	7·0	95·8
24th to 30th "	10	19·6	163·5
31st to 6th January	12	28·0	230·9
7th to 13th "	16	38·6	318·5
14th to 20th "	24	97·0	551·9
21st to 27th "	48	87·6	672·0
28th to 3rd February	51	149·8	848·4
4th to 10th "	92	203·0	997·4
11th to 17th "	124	282·5	1201·0
18th to 24th "	178	261·4	1334·2
25th to 3rd March	280	328·1	1320·6
4th to 10th "	387	398·7	1245·6
11th to 17th "	442	416·6	1297·6
18th to 24th "	644	404·6	1166·2
25th to 31st "	779	314·7	1021·9
1st to 7th April	702	248·2	687·4
8th to 14th "	695	321·6	763·7
15th to 21st "	870	432·5	698·6
22nd to 28th "	925	294·1	437·1
29th to 5th May	802	171·6	250·8
6th to 12th "	578	158·8	173·6
13th to 19th "	404	121·6	126·0
20th to 26th "	296	52·6	91·6
27th to 2nd June	162	54·6	75·2
3rd to 9th "	106	28·2	33·8
10th to 16th "	64	11·7	37·3
17th to 23rd "	46	17·7	36·2
24th to 30th "	35	19·6	29·4
1st to 7th July	27	15·2	31·5
8th to 14th "	28	9·3	15·2
15th to 21st "	24	15·3	25·8
22nd to 28th "	26	9·4	32·7
29th to 4th August	29	19·6	26·6
5th to 11th "	17	16·8	36·4
12th to 18th "	31	20·3	44·4
19th to 25th "	15	19·6	33·6
26th to 1st September	14	31·6	42·1
2nd to 8th "	18	18·7	52·5
9th to 15th "	22	36·6	36·6
16th to 22nd "	22	30·8	47·6
23rd to 29th "	30	21·0	35·0
	177	102·0	323·9

are separated only by a short interval of time, the plague season and the off-plague season, as they may conveniently be termed, are for practical purposes the same for both. The off-plague season extends roughly from June to November inclusive, the plague season occupying the remaining months of the year. It will, however, be noted that acute plague was found in both *rattus* and *decumanus* in Bombay City in every week throughout the whole year.

(b) *The relation in point of time of the decumanus epizootic to the rattus epizootic.*

This is well seen in the chart for Bombay City (III.). It will be noted that the *decumanus* curve as it ascends crosses the mean line at a point corresponding to the week January 7th—13th, while the *rattus* curve crosses the mean line about the end of January.

The *decumanus* epizootic was at its height approximately between February 21st and March 14th, while the corresponding stage of the *rattus* epizootic was about ten days later, namely, approximately March 7th to March 21st.

The curve for *Mus rattus* shows a second maximum in the week April 15th—21st, the corresponding point in the *decumanus* curve occurring in the previous week. It is obvious then that the *decumanus* epizootic precedes the *rattus* epizootic by a mean interval of about ten days.

This is further apparent by a general study of the corresponding curves made for several of the sections into which Bombay is divided.

THE EPIZOOTICS IN RELATION TO PLACE.

(a) *The diffusion of the infection.*

It might be expected from the account we have already given of the habits of the two species that *Mus decumanus* is more important in this connection than *Mus rattus* on account of its out-of-door life and wandering habits. A study of the maps of the sections<sup>1</sup> confirms this view and leads us to the conclusion that *Mus decumanus* is chiefly responsible for the diffusion of the infection amongst the rats throughout the City.

<sup>1</sup> Monthly spot maps showing the place incidence of *decumanus*, *rattus* and human plague were prepared for all the sections of Bombay City. One set (that of Fort North and South, see Map I.) are reproduced in Appendix I.

In the early months of the epizootic only a few *Mus decumanus* appear on the maps. Each of these doubtless represents a focus for the spread of the infection, because the succeeding maps show local extensions of the infection amongst *Mus decumanus*.

The question arises as to whether extension of infection from the badly infected sections to neighbouring little infected sections takes place. It has, we believe, been suggested that the epizootic usually begins in Mandvi (*vide* map of Bombay)—a notorious plague spot—and spreads in a peripheral manner to the outlying sections. We can find no evidence for this view; indeed a study of the maps and charts entirely opposes it. In Table X. the week corresponding to the climax of each epizootic is given for a certain number of sections. The dates are only approximate, but they show that a definite extension from one section to another is certainly not the rule. Fort North in the Fort section is an interesting example of a badly infected area, isolated from another badly infected section, Mandvi, by a section (Esplanade), which is notably exempt from plague, and yet the epizootic in Fort North and Mandvi followed practically the same course throughout.

TABLE X. *Showing weeks corresponding to height of epizootic in various Sections, as judged by the weekly rat figures corrected for a week of 7 days.*

Locality	Date of climax of <i>decumanus</i> epizootic	Date of climax of <i>rattus</i> epizootic
Bombay City	(1) February 18—24	(1) March 11—17
except F and G wards	(2) April 8—14	(2) April 15—21
Mandvi	February 18—24	March 4—10
Chakla	February 25—March 3	March 11—17
Oomarkhadi	February 4—10	February 11—17
Dongri	February 18—24	March 4—10
Market	March 18—24	March 25—31
Khara Talao	February 4—10	February 18—24
Khumbharwada	March 11—17	March 25—31
Khetwadi	March 18—24	April 8—14
Fort North and South	February 25—March 3	March 25—31
		April 15—21

Moreover, the supposition that the infection spreads in a definite manner from one section to another is rendered unnecessary by the fact, that infection amongst the rats persists during every month of the year in most of the sections.

Lastly, the question of the migration of rats as a factor in the spread of the epizootic may be adverted to. The term, migration, seems to have been used in this connection to mean, that a general movement

sets in amongst the rats in a place when plague breaks out amongst them. We can only state that we have failed to find the slightest evidence from our observations in Bombay City and in the outlying villages, which might lend support to this idea.

(b) *The severity of the epizootics.*

The infection amongst the rats of Bombay must be characterised as being exceedingly severe. Table VI. gives sufficient proof of this, but the following statements will enable the reader readily to realise the extreme severity of the epizootics: 4381 *Mus rattus* and 13,277 *Mus decumanus* were shown by us to be plague infected during the year: a total of nearly 18,000 plague rats out of a total of 117,000 rats examined. The largest number of infected *Mus rattus* for one week was 432, the largest number of *Mus decumanus* for a similar period being 1334.

The largest number of plague rats obtained in one day was 277. On 12th March, 1906, a total of 296 dead *Mus decumanus* were examined and of these 148 were plague infected, i.e. 50 %. On 20th March, 1906, a total of 219 dead *Mus rattus* were examined and of these 89 or 40 % proved to be plague infected.

Out of every five *decumanus* examined for the whole year, one was infected, and similarly one out of every ten *Mus rattus*.

The total number of plague rats sent from Mandvi during the year represents an average of 3·1 plague rats for every building in this section. In at least eight sections the average number per building (calculated on the total buildings in each section) ranges from 0·1 to 3·1 plague rats (Table XI.). Mandvi heads the list of the sections with a total for the year of 1065 infected *Mus rattus* and 1808 infected *Mus decumanus*, a total of 2873 plague rats. The comparative severity of the epizootics in the various sections is illustrated in Table XII. Mandvi again has the unenviable distinction of coming first in this Table.

It will be seen that plague rats were obtained every week throughout the year from Mandvi, that infected *Mus decumanus* were obtained also every week and that in only three weeks in the year were no plague *Mus rattus* found in this section. It would seem evident that Mandvi is the worst section in the City as regards the severity of the epizootic.

The widespread character of the epizootic was excellently illustrated in the maps for the sections; maps of one section are reproduced.



TABLE XI. *Showing comparative severity of epizootic and epidemic in various sections.*

Sections	Average number of plague deaths per building	Average number of total plague rats per building	Plague deaths per mille
Dongri	1.0	1.9	16.4
Mandvi	0.6	3.1	14.4
Khara Talao	0.6	1.9	12.1
Bhuleshwar	0.6	1.6	17
Khumbharwada	0.6	1.6	11
Oomarkhadi	0.5	1.7	10.7
II Nagpada	0.5	1.0	11.2
Kamathipura	0.5	0.6	13.1
Dhobi Talao	0.5	1.2	14.2
Girgaum	0.3	0.1	9.4
Khetwadi	0.2	0.2	8.0
I Nagpada	0.2	0.0	3.6
Esplanade	0.1	0.08	6.7
Mahalakshmi	0.08	0.01	5.8

TABLE XII. *Showing comparative severity of epizootics in various sections.*

Sections	No. of weeks no plague <i>rattus</i> were obtained	No. of weeks no plague <i>decumanus</i> were obtained	No. of weeks neither plague <i>rattus</i> nor plague <i>decumanus</i> were obtained	No. of weeks there were no plague deaths
Mandvi	3	0	0	4
Oomarkhadi	9	3	1	12
Dongri	13	2	1	8
Chakla	19	6	4	19
Khara Talao	14	4	4	21
Bhuleshwar	17	10	6	11
Fort North & South	20	11	6	13
II Nagpada	26	12	10	23
Khumbharwada	27	14	12	25
Kamathipura	23	17	12	15
Dhobi Talao	27	15	14	16
Market	26	22	16	16
Colaba	41	23	22	—
Fanaswadi	32	24	23	17
Byculla	37	26	25	16
Khetwadi	36	32	31	23
Mazgaon	42	37	34	19
Tardeo	41	40	34	29
Esplanade	41	37	34	32
Girgaum	42	37	36	28
Tarwadi	45	44	41	16
Chowpatti	49	42	42	30
Mahalakshmi	48	45	43	31
Walkeshwar	49	46	46	31
I Nagpada	52	52	52	43

*(c) Severely infected foci.*

Such a focus is well exemplified in an outbreak which occurred amongst *Mus decumanus* in Samuel Street and De Souza Street in Mandvi. In these streets there are a large number of gunny bag godowns<sup>1</sup>, a class of godown which is particularly favoured by rats on account of the shelter they afford and on account of the residue of grain in the sacks.

Again, certain gullies in Oomarkhadi section show a severe infection amongst the rats, especially amongst *Mus decumanus*. In January, for example, 49 plague rats, of which 47 were *Mus decumanus*, were obtained from two of the gullies.

*(d) The persistence of infection in localities.*

A good example of the long persistence of infection in a localised area was noted in Mandvi section in the neighbourhood of De Souza Street. The first plague rat found was a *Mus rattus* on 19th September, 1905, the date of finding the last rats (two *Mus decumanus*) being 29th December, 1905, an interval of at least three months.

*(e) The question of the re-infection of houses or buildings by rats.*

This is a matter of some importance in relation to prophylaxis. Our data with regard to it are, however, meagre. In an outbreak of plague in Sion Koliwada village we could obtain no evidence that re-infection occurred amongst the rats (*Mus rattus*) in the houses attacked early. In Bombay City it is possible that re-infection of the house rats in the plague season may take place on account of the widespread and severe infection amongst *Mus decumanus* and the association of this rat with *Mus rattus*.

### III. THE RELATION OF THE DECUMANUS EPIZOOTIC TO THE RATTUS EPIZOOTIC.

In the consideration of this question a study of the charts and maps for Bombay City and for the sections is essential. On the charts three curves will be noted which in order of time represent the *decumanus* epizootic, the *rattus* epizootic and the human epidemic. Each of these curves is separated from the next by a space representing an interval of time, the interspaces, and therefore the time intervals, being very nearly the same. (See Charts III.—XV.)

<sup>1</sup> Gunny bags are used chiefly for grain; the empty bags are stored in these godowns.

We may state without discussion in this place that the sum of the evidence as amassed by us leads us to the conclusion that the human epidemic is due entirely to the epizootic amongst the rats, and that of the two epizootics the *rattus* epizootic must be held directly responsible for the epidemic. Accepting this as a just conclusion it naturally suggests itself that similarly the *decumanus* epizootic is directly accountable for the *rattus* epizootic.

In this statement we merely wish to express the general relation between the two epizootics. We do not suppose that in every instance *Mus rattus* received its infection directly from a plague-infected *Mus decumanus*, because we know that when infection is introduced into a colony of *Mus rattus* the presence of infected *Mus decumanus* is not essential for its continuance.

It seems to us from the following considerations that it is impossible to escape from the conclusion mentioned above.

There can be no doubt that the *decumanus* epizootic precedes the *rattus* epizootic in time. The maps of the sections bring this out very well. Since, however, each map represents a period of one month, the charts for the sections (fortnightly periods), and especially that for Bombay City (weekly periods), express the time relations more accurately.

It is quite evident from the latter chart that a definite interval exists between the *decumanus* epizootic and the *rattus* epizootic, the mean interval of time being about ten days.

If now the interval between the two epizootics corresponds with the mean interval of time, which from actual observation is found to elapse between the exposure to infection of *Mus rattus* and its death, we shall have good grounds for concluding that the *decumanus* epizootic is responsible for the *rattus* epizootic. The following direct evidence is very important in this connection. Thirty successful flea transmission experiments were carried out in the laboratory with *Mus rattus*. The method employed was to transfer to healthy *Mus rattus* in flea-proof cages infected fleas which had fed on septicaemic rats. The fleas were transferred from these animals after their death from plague to the healthy *Mus rattus*. The number of days from the date of exposure to the bites of the fleas to the death of *Mus rattus* has been computed as accurately as possible and an average has been struck. The average for the thirty experiments is 7.2 days.

This correspondence of the experimental mean with the mean expressed in the chart, when taken in conjunction with the fact that

the *decumanus* epizootic precedes the *rattus* epizootic, appears to us to be strong evidence in support of the conclusion that the *decumanus* epizootic stands in a causal relation to the *rattus* epizootic<sup>1</sup>. We have already pointed out that the condition for epidemic spread (*i.e.* prevalence of fleas) is greater in *decumanus* than *rattus*; hence it is not difficult to imagine why the *decumanus* epizootic precedes that in *rattus*.

#### IV. PLAGUE IN *MUS MUSCULUS*, *NESOKIA BENGALENSIS* AND *NESOKIA BANDICOTA*.

*Mus musculus*. Since no special care was taken in the collection of mice we can make no definite statement as to the severity of the infection amongst them. Only 63 infected mice were obtained during the year. The regional distribution of the buboes and the post-mortem appearances generally corresponded with these features as met with in plague rats.

*Nesokia bengalensis*. The remarks made above with regard to mice apply equally in the case of this rodent. Peripheral buboes were commonly present and were similar in distribution to those in rats, and the pathological appearances, including "granular" liver and pleural effusion, were identical with those met with in plague rats.

*Nesokia bandicota*. Very few specimens of plague-infected bandicoots have come to our notice.

*Musk rat*. It is interesting to note that we have never met with a specimen of a plague-infected musk rat, although on account of the habits of this animal it must frequently be exposed to infection. The explanation is simple, namely that the musk rat is highly resistant to plague, as we have proved by experiment. It withstands the subcutaneous inoculation of as large a dose as  $\frac{1}{5}$  of an agar-tube culture of a virulent strain of the bacillus.

<sup>1</sup> The curves for the epizootics may for practical purposes be said to be calculated on dead rats since the numbers of live rats found infected formed a very small proportion of the total infected rats. The experimental result may therefore with fairness be compared with the time as shown in the Chart. Further, in the experiments the fleas were always fed on a rat which had a large number of bacilli in the blood and a considerable number were used in each experiment. Both these factors would tend to shorten the interval between the death of the rats, in comparison to what occurs in nature.

V. SUMMARY.

It has been shown that *Mus decumanus* and *Mus rattus* are by far the most important species of rodent in Bombay in relation to the spread of plague. A short account has been given of the external appearances which we relied upon in distinguishing between the two species. It has been pointed out that *Mus rattus* is essentially a house rat, that it is very numerous in the native houses, and that it has a universal distribution in Bombay Island. *Mus decumanus*, although typically an out-of-door wandering rat, is yet found not infrequently in the lower floors of inhabited buildings, and is practically confined to Bombay City. The importance of the close association of the two species in certain common haunts lies in the relation of the *decumanus* epizootic to the *rattus* epizootic.

*Mus decumanus* does not occur in the outlying villages in the Island, a circumstance which is referable to the absence of gullies, drains, etc. in these villages.

*Nesokia bengalensis*, a rodent closely resembling *Mus decumanus*, accounts for about 1% of the rodent population in the City: it is susceptible to plague.

With regard to the epizootic amongst the rats the following conclusions may be formulated:—

(1) *Mus decumanus* and *Mus rattus* are equally susceptible to plague.

(2) The incidence of plague is twice as great on the *decumanus* population as on the *rattus* population.

(3) *Mus decumanus* is the species which is chiefly responsible for the diffusion of plague amongst the rats throughout Bombay City.

(4) The *decumanus* epizootic precedes the *rattus* epizootic by a mean interval of about ten days.

(5) The *rattus* epizootic is directly attributable to the *decumanus* epizootic.

(6) Plague persists in the rats in Bombay City during the off season. This persistence is due chiefly to *Mus decumanus*.

It seems to us that the last four conclusions can best be correlated, and are adequately explained if it be granted that the conditions for epizootic prevalence are more favourable in the *decumanus* population than in the *rattus* population.

The only factor concerned in the severity of epizootic prevalence, which, so far as our knowledge extends, does not affect the two species



equally, is the degree of flea-infestation of each, *Mus decumanus* harbouring more than twice as many fleas as *Mus rattus*. We think, therefore, that in this fact is to be found the key to the elucidation of the relationship of the two epizootics.

## V. THE EPIDEMIC AND ITS RELATION TO THE EPIZOOTICS.

### I. GENERAL ACCOUNT OF THE EPIDEMIC OF 1905-6.

#### (1) *Statistical data relating to the severity of the epidemic.*

The epidemic of the year under review, when compared with the yearly outbreaks in the City dating from 1897, may be described as being of moderate severity. 12,245 attacks were reported during the year from the whole of the island. Of these 74 were "imported" cases, *i.e.* a history was obtained that they had recently arrived in the island from an infected locality, where presumably they had received their infection. 11,010 plague deaths were reported during the year. Data concerning 10,880 plague cases, or 89% of the reported attacks, were entered into the case cards in accordance with the system of which we have already given a description. It was found impossible towards the climax of the epidemic to keep pace with the large amount of work involved in filling up the case cards. In spite of this nearly all the sections are well represented in the cards.

Deaths from plague were reported from the City during every week of the year, the smallest number—five deaths only—being in the week 19th—25th November and the largest number—925—in the week 22nd—28th April. The largest number of plague deaths in one day was 167, *viz.* on the 30th April 1906. The largest number of deaths in the year for a single section occurred in Byculla (917). Only in four weeks during the year were no plague deaths reported from Mandvi. The death rate from plague was greatest in Bhuleshwar section, in which it reached the high figure of 17 per mille of the population of this section. In 19 sections out of 25 the death rate rose above 10 per mille of the population of each section.

(2) *Statistical data relating to the incidence of plague on the population, classified according to sex, age and religion.*

Our data on these points, obtained by an analysis of the case cards, are set forth in Tables XIII., XIV. and XV.

(a) With regard to the incidence on sex it might appear at first sight that males are somewhat more liable to infection than females.

TABLE XIII.

*Incidence of plague on the two sexes.*

No. of males attacked by plague	No. of males in population (census 1906)	Ratio of infected to non-infected males
7,211	612,965	1—85
No. of females attacked by plague	No. of females in population (census 1906)	Ratio of infected to non-infected females
3,669	364,811	1—99

TABLE XIV.

*Incidence of plague on persons of different age-periods.*

Age-period	No. of attacks for each age-period	No. of population in each age-period (census 1906)	Incidence per mille for each age-period
0—5	239	69,775	3·4
6—10	878	70,009	12·6
11—20	3,519	187,321	18·7
21—40	5,102	478,101	10·6
41—60	1,040	142,715	7·2
Above 60	102	26,574	3·8
Total	10,880	974,495	—

TABLE XV.

*Incidence of plague on persons of different religions.*

Religion	Numbers attacked by plague	No. of each religion in the population (census 1906)	Incidence of plague per mille
Brahmins	456	706,154	12·4
Jains	15		
Other Hindus	5,626		
Low caste Hindus	2,724		
Parsees	310	48,824	6·3
Mahomedans	1,414	168,677	8·3
Native Christians	263	48,508	6·1
Eurasians	14		
Europeans	19		
Jews	32	5,367	5·6

We do not think that this is actually the case, because it is well known that in Bombay concealment of cases amongst females, especially among Mahomedan women, is more largely practised than amongst males. It seems to us, therefore, very probable that little difference exists in the liability to infection of persons of either sex.

(b) Table XIV. shows that there are marked variations in the incidence of plague on persons of different ages. The incidence increases from birth to the age of 20 and afterwards diminishes. The greatest incidence falls on persons in the age-period 11—20. It would seem difficult to explain these variations on grounds other than a varying susceptibility to the disease at different age-periods.

(c) Table XV. shows that Hindus (including Jains) are most liable to infection. Mahomedans come next in order and are followed by Parsis and a group which includes Native Christians, Eurasians and Europeans. The incidence appears to be least in Jews. The most probable explanation of this variation appears to us to be found in the conditions of life (poverty, habits, etc.) which, as we shall see, greatly influence the liability of the people to exposure to infection from rats.

## II. THE RELATION OF THE EPIDEMIC TO THE EPIZOOTICS WITH REGARD TO TIME AND PLACE.

### (1) *The time-relations of the epidemic and the rattus epizootic.*

The charts for Bombay City and for the sections show that the epidemic curve has a marked similarity to the curves which represent the epizootics. The seasonal prevalence of the epidemic therefore corresponds to that of the epizootics.

The epidemic curve for Bombay City crosses the mean line in the week 18—24 February. The interspace at this point separating the epidemic from the *rattus* epizootic represents an interval of  $3\frac{1}{2}$  weeks. As the epidemic curve rises towards a summit in the last week of March it approximates to the epizootic curve so that when it reaches this summit the interval is reduced to a fortnight. The epidemic curve shows a second well-marked apex which is separated by only a week's interval from the corresponding apex of the *rattus* epizootic. The mean interval between the epidemic and the *rattus* epizootic curves may therefore be said to be approximately 10 to 14 days. This result is further borne out by a study of the curves for the separate sections.

If the epidemic is directly attributable to the *rattus* epizootic, it

becomes necessary to furnish an adequate explanation of the interval of time which elapses between the epidemic and this epizootic. It must be pointed out that this interval, if our conclusions are correct, must be capable of being interpreted, on the view that the rat flea is the transmitting agent of the infection from rat to man. On this view our explanation of the mean interval expressed in the charts is as follows. We consider that this interval is divisible into three periods: (a) a period, which elapses between the death of an infected rat and the communication of the infection from the rat to man by the rat flea, (b) the incubation period of the disease in man, and (c) the period of duration of illness in a fatal case of human plague.

(a) From observations<sup>1</sup> we have made it would appear that when an opportunity is offered to rat fleas of biting man the best results are obtained when the fleas have starved for about three days, *i.e.* under these conditions the largest number of fleas are found to bite man. The apparent disinclination of the rat flea to attack man, even when he is the only available host, is naturally explained by the fact that man is not the true host of *Pulex cheopis*.

(b) The best evidence relating to the average length of the incubation period in human plague has been collected by the Indian Plague Commission and is presented in their report (vol. v. p. 77). From this evidence it would appear that the mean incubation period approximates to three days.

(c) The mean duration of illness in fatal cases of plague may be stated to be  $5\frac{1}{2}$  days. This result has been arrived at as follows: the mean duration of illness of 100 fatal cases reported on the case cards is 3·6 days. The duration of illness in 64 hospital cases, who died from the disease and from whose blood we recovered the *B. pestis*, works out at 7·5 days. The mean of these figures is 5·5 days, so that we may regard the mean duration of illness in a fatal case as approximately  $5\frac{1}{2}$  days.

Summarising the above data we obtain the following results:

Time elapsing before the rat flea bites man	...	3 days.
Incubation period of human plague	... ..	3 ..
Duration of illness of fatal human plague	... ..	$5\frac{1}{2}$ ..
		<hr/> 11 $\frac{1}{2}$ ..

It is evident that the mean period of 11 $\frac{1}{2}$  days corresponds fairly satisfactorily to the mean interval of time expressed in the charts.

<sup>1</sup> These observations will be published in a later volume.

The time-relations of the epidemic and the *rattus* epizootic are well illustrated in the maps for the sections.

(2) *The place-relations of the epidemic and the rattus epizootic.*

The direct evidence bearing upon this point which we have been able to collect in Bombay is, from unavoidable causes, meagre. We were able to make a search for dead rats in only a small proportion of the total houses and buildings in which plague cases occurred. Still, in a considerable number of instances plague-infected *M. rattus* have been found in houses in association with plague cases.

From indirect evidence, however, there can be not the slightest doubt that the place-infection of man is intimately related to that of *M. rattus*. We refer to the fact—a commonplace doubtless to workers in India, but not perhaps sufficiently appreciated by those who have not visited this country—that *M. rattus* is essentially a house-rat and that it lives in close association with man. It necessarily follows from this association that the place-infection of *M. rattus* must correspond closely to that of man in the sense that both must be referred to inhabited buildings.

(3) *The quantitative relation of the incidence of human and of rat plague.*

This is well shown for any one section in the charts of the sections, *i.e.* the epidemic and the epizootics increase and diminish in severity *pari passu*.

In order to contrast one section with another, from this point of view, Table XI. should be consulted. It will be observed that the first nine sections in this table show an incidence for human plague deaths of over 10 per mille of the population of each section, corresponding to an average of 1 to 3·1 plague-infected rats per building in eight of the sections. In the last five sections in the table, in which the human plague incidence is considerably less, the average number of total plague rats per building is also considerably smaller. Confirmatory evidence is given in the column in this table, in which is set forth the average number of plague deaths per building. Although the figures for the rats are only approximate the figures in the table taken as a whole certainly indicate a quantitative relation between the severity of the epidemic and the severity of the epizootics in the different sections.



(4) *Further remarks on the spread of infection from rat to man.*

In an account of the epizootic we have arrived at the conclusion that the *rattus* epizootic is directly attributable to the *decumanus* epizootic. At the same time it was pointed out that in this statement we do not wish to imply that in every instance *M. rattus* received its infection directly from *M. decumanus*. Further, from the considerations which have been brought forward in the present discussion, we think it justifiable to conclude that the epidemic is directly attributable to the *rattus* epizootic.

It would seem impossible, however, to escape from the conclusion that *M. decumanus* occasionally transfers infection directly to man, in view of the fact, to which we have already alluded, that *M. decumanus* is to a certain extent a house-rat. Since *M. decumanus* is not, however, so generally distributed over buildings as *M. rattus*, we do not think that direct infection of man by the former species occurs nearly as frequently as it does by the latter species of rat. The charts unquestionably support this conclusion, since they show that the epidemic is more nearly related in time to the *rattus* epizootic than to the *decumanus* epizootic.

### III. SUMMARY AND CONCLUSIONS.

With regard to the incidence of plague on different classes of the population we may note that little difference, if any, exists in the liability to infection of males and females; that there is a varying incidence on persons of different age-periods, the greatest incidence being on persons between 11—20 years of age; and that of the different races in Bombay Hindus and Mahomedans suffer most severely from the disease.

We may summarise our conclusions regarding the inter-relations of the epidemic and the epizootics as follows:

(1) The time-relation of the epidemic and the *rattus* epizootic is explicable on the view that the rat flea is the transmitting agent of the infection from *M. rattus* to man.

(2) From the point of view of place-infection there is an intimate relation between the epidemic and the *rattus* epizootic.

(3) There is a definite quantitative relation between the incidence of human and of rat plague.

(4) The epidemic is directly attributable to the *rattus* epizootic and since this epizootic is in its turn directly attributable to the

*decumanus* epizootic, the epidemic is indirectly attributable to the latter epizootic.

While the last conclusion expresses the broad relations of the epidemic and the epizootics it must be added that:

(5) Infection is occasionally transferred directly from *M. decumanus* to man, i.e. without the intervention of *M. rattus*.

## VI. THE SANITARY CIRCUMSTANCES IN BOMBAY CITY WHICH INFLUENCE THE SPREAD OF EPIDEMIC PLAGUE.

- I. Introduction.
- II. A. The buildings in the native city and their construction.  
B. The relation of these conditions to the spread of plague.
- III. The collection, removal and disposal of excretal and other refuse.
  - 1. The relation of the sanitary arrangements to rat infestation.
  - 2. General considerations.
- IV. Overcrowding of the population.  
The question of the relation of overcrowding to the spread of plague.
- V. The habits and customs of the people.  
The habits of the people in relation to rat infestation.
- VI. The social condition of the population.
- VII. Summary.

### I. INTRODUCTION.

Having arrived at the conclusion that the epidemic is dependent upon the epizootics, we may proceed to inquire what the conditions are in Bombay which favour the spread of epidemic plague.

The inquiry resolves itself mainly into a consideration of the conditions in Bombay which favour the spread of epizootic plague and which facilitate the transference of infection from rats to man. While this is so, it is impossible to disregard the point of view of those who maintain that certain insanitary conditions (considered without reference to rat plague) play an important part in the spread of the epidemic. A brief description of the principal sanitary defects in the City will, therefore, be given, and discussion made as to their influence on plague. As a matter of fact several of these defects, in our view, have an important influence on human plague, for the sole reason, however, that they promote rat infestation and thus favour the spread of epizootic plague. Since frequent reference will be made in the following pages to the subjects of rat infestation and of rat plague, we would point out

that it will conduce to a clear understanding of the issues involved in the whole problem, if the reader will distinguish between (1) the conditions which favour the spread of rat plague over the city (apart from its spread into buildings); (2) the conditions which favour the spread of rat plague into, and throughout, inhabited buildings; and (3) insanitary conditions (without reference to rats), which, as has been alleged, increase the liability to infection of man and at the same time aid in the spread of human plague.

We propose in the account that follows to describe, in the first place, the prevailing types of buildings in Bombay City, together with the conservancy arrangements in connection with such buildings. We shall, then, give a description of the conditions in which the people who occupy these buildings live, making special reference to the question of overcrowding, of the social conditions of the people and of their habits and customs. Lastly, the bearing of these points upon the spread of the epizootics and the epidemic will be discussed.

Before concluding these introductory remarks a word of explanation is necessary as to the nature of the evidence which we shall bring forward in support of our conclusions. This evidence is of two kinds. First, in the two years during which the Commission has been working in Bombay ample opportunity has been offered to its members for personal observation of the various conditions which prevail in the native city. Full advantage has been taken of these opportunities, so that from personal observation and experience we have been enabled to form definite opinions on the problems which presented themselves. Secondly, we have collected a large mass of statistical data in the manner already described. We are aware that these data are not altogether free from fallacies. Attention has already been drawn to certain sources of error, which in an oriental city like Bombay cannot fail to affect the accuracy of statistics relating to human plague.

## II. A. THE BUILDINGS IN THE NATIVE CITY AND THEIR CONSTRUCTION.

### 1. *General description.*

The typical inhabited building in the native city is a tenement building of considerable size; it is separated from adjoining buildings by gullies. The average size of the buildings is indicated in the statement that the average number of inhabitants per building for the whole island is about 30. Although the majority of the buildings shelter less

than 20 persons, yet there are a considerable number with over 50, several with 200 and even a few with over 400 inhabitants.

The buildings are divided into houses or tenements, each holding being occupied as a rule by a single family. In some instances, however, we have found several families in one room, the number varying according to the size of the room. The majority of the buildings in Bombay are subdivided into ten or less holdings, but there is a considerable number containing 50 houses and over. In this connection it is worthy of note that 87·5 % of the houses or holdings consist of one room only, and that 85 % of the population live in such houses. This statement gives a good idea of the poverty of the inhabitants generally.

Attention may be drawn here to a type of building, known as a "chawl," which is common in some sections in Bombay, and which may be described as a large tenement building occupied by the poorer class of natives. We may add that most of these chawls embody the worst structural features of buildings in Bombay. Plate XXIII shows good examples of this type of building.

One of the commonest, and at the same time one of the most important, characteristics of the buildings in Bombay is, that the ground-floors are often occupied with shops, godowns, or even stables. These shops, of course, vary greatly as to the nature of the articles exposed for sale, but most frequently, perhaps, they contain grain and other articles of food.

In Mandvi the ground-floors are very commonly occupied by godowns (Plate XXV), at least 70 % of the buildings in this section showing this peculiarity. The nature of these store-houses or godowns varies, but the commonest commodities stored in them are grain, spices and gunny-bags (empty grain sacks). Buildings with stables on the ground-floor are found everywhere.

## 2. *The construction of the buildings.*

The buildings in Bombay are for the most part of a flimsy construction. Proper foundations do not exist; the walls rest upon a plinth composed of rubble and earth with an outer facing of stone. The floors of the basement consist merely of the soil over which the building is constructed, rendered firm by a layer of rubble and earth and covered with a layer of beaten earth.

(a) The *walls* consist of a framework of stout wooden beams, which support the upper storeys, the spaces between the beams being filled up



770



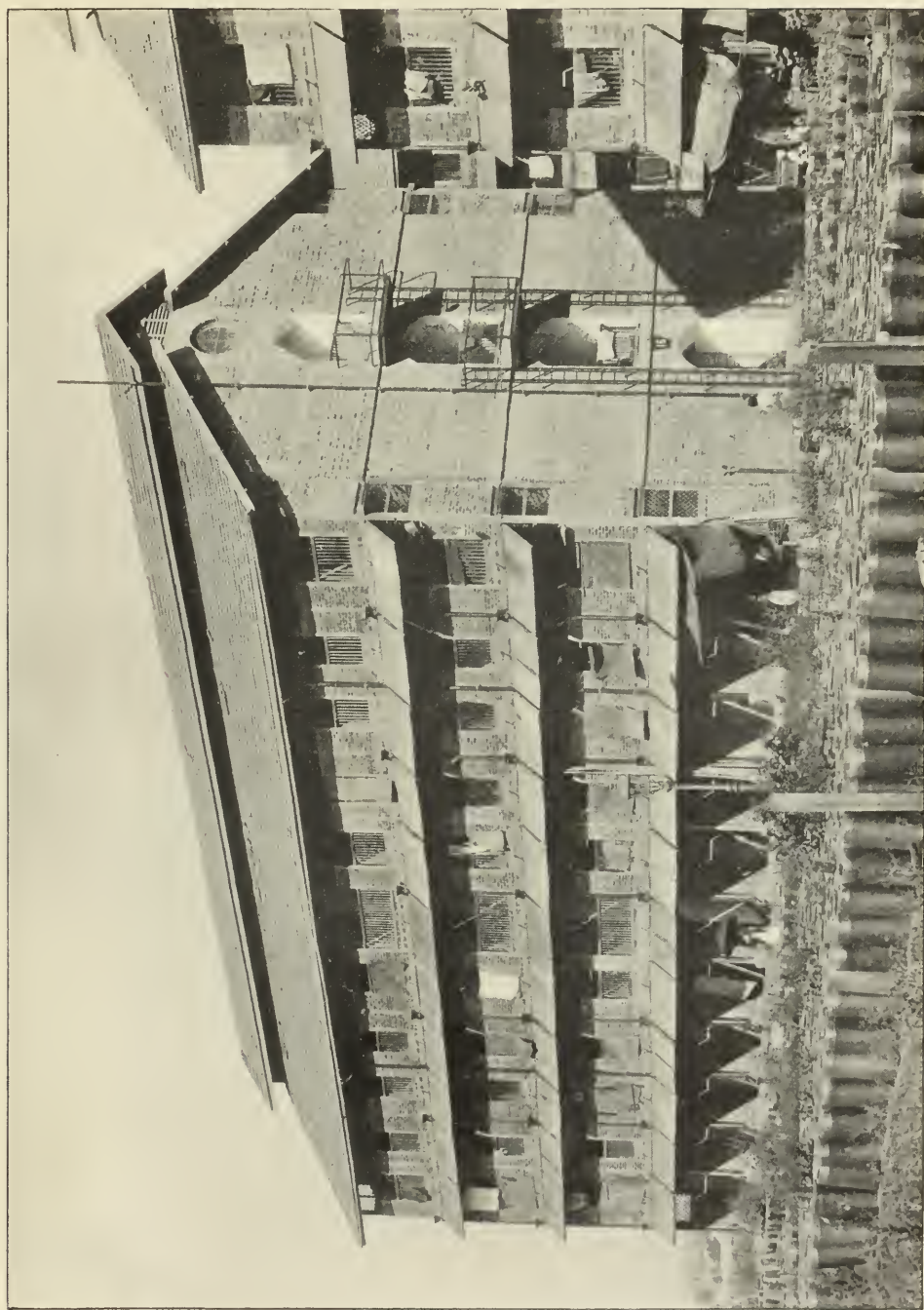
Bombay City: showing a typical chawl.



Bombay City: a typical chawl.







Bombay City: showing one of the model chawls in Morland Road which was badly infected with Plague.





Bombay City: showing country tiles on roofs and a gully between the houses.



Bombay City: showing go downs on ground floor.





with burnt bricks and mortar. The outer and inner surfaces of the walls are plastered over with a layer of "chunam"—a mixture of lime and sand—put on moist and allowed to set and then ornamented with some coloured wash.

(b) The *floors* are formed of boarding supported on joists. Such a simple wooden floor is, however, very rarely seen in Bombay. The planking is very frequently covered with a layer of beaten earth, on the surface of which cowdung is periodically applied. The earthen floor in a good many instances is overlaid with a layer of "chunam." On the ground-floor the boarding is, as a rule, dispensed with.

(c) The *roofs* consist of wooden joists, supporting a lattice of laths, on which are laid two to four layers of country tiles (Plate XXV). The great majority of buildings have this form of tiling, but in a certain number a single layer of flat Mangalore tiles is used (Plate XXIV). The tiled roofing generally serves the purpose of a ceiling in the case of rooms on the top storeys of buildings, that is to say, there is no proper ceiling in these rooms.

(d) *Ventilation* and *lighting* are provided for by means of the door, which invariably fits loosely, and by windows which, however, are sometimes absent. The windows consist of an upper and lower portion. The lower portion, in the majority of cases, is fitted with an iron grid and is capable of being closed by a wooden shutter. The upper half in the better class of houses is fitted with glass panes and is also provided with shutters. The windows sometimes look out upon dark and narrow gullies. From this description it might be imagined that the ventilation in the houses must be miserably deficient. It ought to be remembered, however, that the walls are flimsy, that the tiled roofs act as excellent ventilators, that the doors and the window-shutters fit very loosely and that, except in the colder nights of January and February, the windows are kept open.

In spite, therefore, of the overcrowding, which according to our British standard undoubtedly exists, we are of opinion that the ventilation in the great majority of the houses is adequate. We may note in support of this opinion, that the houses in Bombay rarely give the impression of closeness which is so common a feature in the houses of the poor at home.

The houses of the poorer class of natives are not infrequently badly lighted. It is not uncommon to observe the verandahs and windows covered up with screens of matting, pieces of cloth, etc. Some of the houses, from structural defects, are so dark that the occupants resort to

lamplight during the day time. The corridors in chawls are also often very badly lighted.

B. THE RELATION OF THESE CONDITIONS TO THE SPREAD  
OF PLAGUE.

1. *The construction of the buildings.*

In an account of the epizootic we have already given details concerning the rat infestation of buildings.

It has been shown that *Mus rattus* is found in all parts of buildings, while *Mus decumanus* can be trapped only on the lower floors.

It is not difficult to connect these facts with the generally flimsy construction of the buildings as described above. We have, indeed, very frequently observed the floors and walls of houses riddled with the holes and burrows of *Mus decumanus* and of *Mus rattus*. In particular, we would point out that the inroads of *Mus decumanus* into buildings are greatly favoured by the defective plinths and by the lack of rat proof foundations. Rat holes may frequently be observed between the stones of the plinths and we have seen the material of which the foundations are made riddled in all directions with rat burrows.

We have not been able to obtain any statistical data bearing upon the relative incidence of plague on this class of buildings and on buildings of a substantial construction. In Bombay relatively few inhabited buildings conform to the latter description. We have, however, on several occasions found plague cases in such buildings. One instance may especially be referred to, in which a serious epidemic took place in a number of chawls, which have recently been built by the Improvement Trust (Plate XXIV). These chawls are of solid structure throughout and indeed in this respect leave nothing to be desired. While, therefore, it is impossible to determine the relative incidence of plague on ill-constructed buildings and on those which are substantially built, we can assert that the latter class of building is by no means exempt from severe plague outbreaks.

2. *The nature of the floors.*

It is obvious that the common type of floor in Bombay, *i.e.* the beaten earth floor, offers no obstacle to a burrowing animal like the rat. We have observed extensive burrows containing the nests of *Mus decumanus* in the floor (overlaid with "chunam") of a living-room in a chawl. In another instance we had the earthen floor (ground-floor) of a room

opened up and found it undermined in every direction with the burrows of *Mus rattus*.

We have no data bearing upon the problem of the relative incidence of plague in rooms with different kinds of floors. We have, however, accumulated figures regarding the nature of the floors in the houses in which 9527 plague cases occurred in the course of the epidemic now under consideration. These figures are set forth in the accompanying table (XVI.), from which it is seen that 57·7 % of the cases were found in houses provided with stone or cement (chunam) floors, 41·5 % in houses with cowdung or earthen floors, and 0·7 % in houses with uncovered wooden floors. While it is impossible to determine the relative frequency of these three types of floors in Bombay, the figures are not without their value, since they show that plague cases very commonly occur in houses with "pucca" floors.

TABLE XVI.

*Showing percentage of plague cases in houses with different kinds of floors.*

	Houses with "pucca" floors	Houses with earth or cowdung floors	Houses with wooden floors	Total
Number of Cases	5495	3960	72	9527
Percentage of total number	57·7	41·5	0·7	

"Pucca" floor includes floors of stone, cement and chunam (namely, a mixture of lime and sand).

### 3. *The nature of the ceilings and roofs.*

Country tiles offer a greater attraction for a climbing rat like *Mus rattus* than Mangalore tiles, simply on account of the shelter provided in the channels of the tiles in the former case. It has been our experience on removing such tiles to disturb many rats and to come across nests with young. The difference in this respect between the two kinds of tiles is well brought out in a table, already published, which shows the rat flea counts in the experimental godowns in the Parel Laboratory (vol. VI. p. 453). It is seen from this table that, summing up the results of several experiments, the number of fleas obtained from guinea-pigs placed in godowns with country tiled roofs is nearly four times the number obtained from guinea-pigs in the godowns with flat Mangalore tiles. Similar observations will be found in an additional paper on experiments in these godowns (vol. VII. p. 421). The explanation of the difference is, as we know from actual observation, that rats frequent the roofs of the godowns with country tiles much more than they do the roofs with Mangalore tiles.

No data are available, which might point to a greater incidence of plague on houses with any particular ceiling or on buildings which are roofed in any particular manner. In the two tables (XVII. and XVIII.) given herewith we see, however, that plague cases may occur under almost any kind of ceiling and in buildings which are roofed either with flat Mangalore tiles or with country tiles.

TABLE XVII.

*Showing percentage of plague cases in houses with different kinds of ceilings.*

	Houses with wooden ceilings	Houses with bamboo, or lath ceilings	Houses with matting ceilings	Houses with corrugated iron ceilings	Total
Number of cases	5150	3412	438	81	9081
Percentage of total cases	56·7	37·6	4·8	0·9	

TABLE XVIII.

*Showing percentage of plague cases in buildings with different kinds of tiles.*

	Buildings with country tiles	Buildings with Mangalore tiles	Total
Number of cases	7603	1185	8784
Percentage of total cases	86·5	13·5	

TABLE XIX.

*Showing percentage of plague cases in houses which differed from one another as regards ventilation.*

	Houses with good ventilation	Houses with fair ventilation	Houses with bad ventilation	Total
Number of plague cases	2915	5221	1491	9627
Percentage of total cases	30·3	54·2	15·5	—

#### 4. *The ventilation of the houses.*

We have already expressed our opinion that the ventilation of the houses in Bombay is not as defective as various writers on the subject have represented. We have not been able to collect statistical data for the purpose of determining whether plague incidence is greater on ill-ventilated than on well-ventilated houses, because we do not know the relative proportions of such houses in the whole of Bombay. Nevertheless, our experience has been, that, while we no doubt came across plague cases in badly ventilated houses, we have likewise seen many cases in houses in which the ventilation left nothing to be desired.

In addition, we can bring forward data obtained from an analysis of the plague case cards, on which the opinion of the inspecting medical



officer regarding the ventilation of plague houses was recorded. The results of this analysis are presented in Table XIX. It is seen that out of 9627 cases the ventilation is classified as good in 30·3%, as fair in 54·2% and as bad in 15·5% of the houses.

#### 5. Defective lighting of houses.

The influence of light and darkness on rat infestation is well illustrated in the godown experiments to which we have just alluded. The flea counts, recorded in the same table, which were made in a country-tile roofed godown with a small roof light, and in a Mangalore-tiled godown, also with a roof light, have been compared with the flea counts obtained in two godowns, similar to the first two but with no roof light. The result is that three times the number of fleas were obtained in the dark godowns as in the godowns with a roof light.

With regard to the relation of the lighting of houses to plague incidence data similar to those obtained for ventilation were recorded on the plague case cards. The result of the analysis of these data is given in the accompanying table (XX.), from which it is seen that the lighting was classified as good in 31·1%, as fair in 53·4%, and as bad in 15·5% of the houses. Moreover, our personal experience of two extensive epidemics in Bombay has been that we have come across many plague cases in houses which were well lighted.

TABLE XX.

*Showing percentage of plague cases in houses which differed from one another as regards lighting.*

	Houses in which the light was good	Houses in which the light was fair	Houses in which the light was bad	Total
Number of plague cases	3002	5151	1493	9646
Percentage of total cases	31·1	53·4	15·5	—

#### 6. Shops, godowns and stables in inhabited buildings.

In an account of rat infestation in Bombay, as evidenced by extensive trapping operations in different situations, it has been shown (Table I.) that relatively to the proportions of the total rats (*Mus rattus* and *Mus decumanus*) caught in all the places where traps were set, more *Mus decumanus* were caught in foodshops than *Mus rattus*, a circumstance which we referred to the proximity of these shops to gullies. On the other hand, food godowns appear especially to attract *Mus rattus* and non-food godowns *Mus decumanus*. Stables have a special attraction for *Mus decumanus*. It must be borne in mind that both species of rats



are found together in all these places. When it is remembered that, as we have shown, the *rattus* epizootic, on which the epidemic directly depends, is in its turn attributable to the *decumanus* epizootic, the significance of the close association of the two species in these common haunts will be at once apparent.

### III. THE COLLECTION, REMOVAL AND DISPOSAL OF EXCRETAL AND OTHER REFUSE.

One of the first things which would attract the attention of a sanitarian on visiting the native city of Bombay is that separating adjoining buildings there is a narrow passage or gully (Plates XXV, XXVI), and that this gully evidently plays an important part in the sanitary arrangements of the buildings.

A gully consists of an open half-pipe channel on each side and an elevated portion in the middle with a small gutter running along its centre. The side channels receive the waste water from the bathing stands in the building. The water passes through an iron screen and trap, which keep back the grosser solid material, into an inspection chamber and then on into the sewers under the street. The central portion of the gully is intended to carry off the storm water in the rainy season. This water, when in full stream, passes over a jump trap at the mouth of the gully and then into a system of storm water drains, which are quite distinct from the sewers. If the stream is a small one, the water falls into the jump trap and thence through the inspection chamber into the sewers.

The gullies also play an important part in the conservancy arrangements of the buildings. In this connection it must be explained that very few buildings in the native town of Bombay are provided with a water-carriage system. When this is present, as it is in the modern buildings, the house-drain is efficiently trapped before opening into the sewer. In the great majority of instances, however, the night soil is removed by hand. In each building there are privies—as a rule on every floor. The privies open into a shoot, which leads down to a chamber on the ground-floor; access to this chamber is obtained from the gully by means of a small door. In the chamber a basket is placed, which receives both the solid and liquid material which comes down the shoot. The liquid material percolates through the sides of the basket into the half-pipe channel at the side of the gully and then flows down this channel along with the sullage water into the sewers. The solid



Bombay City: showing Courtyard with stables.



Bombay City: showing a gully, note the marks indicating the occurrence here of plague-infected rats.



material intercepted in the basket is removed by men called "halalkores" to the nearest central dépôt, where it is emptied into the sewers.

It has been already mentioned that the storm water system of drains is quite distinct from the sewers. The former open into the sea at several points on the shores of the island. They come into complete function only in the rainy season; during the rest of the year they are practically dry.

The proper use of the gullies has already been described. It remains to be added, that the people use them as depositories for all the refuse of the house. From the windows above the sweepings of the rooms, scraps of food and other débris, are freely thrown out into the gully below and to a lesser extent into the streets and lanes. It is the duty of a special corps of municipal servants, known as "sweepers," to keep the streets and gullies clean, but even with their best endeavour this is an almost impossible task.

1. *The relation of the sanitary arrangements to rat infestation.*

The reader will have gathered from the foregoing description that a gully is nothing else than an open drain. Every building in the native city is bounded on at least one side by a gully in communication with the general sewage system and with the storm water drainage system. It is not difficult to understand from this statement that the gully in Bombay is one of the principal haunts of *Mus decumanus*, as well as of *Mus rattus*. The close proximity of gullies to inhabited buildings and the association of the two rats in this situation favour the spread of infection from *Mus decumanus* to the colonies of rats in buildings. Plague-infected *Mus decumanus* are very frequently found in gullies, and we may note that while investigating the infectivity of plague houses plague-infected rats were frequently found a short time before in the adjoining gully.

The food thrown out of the houses into the gullies by the people acts as a continual attraction for rats of both species. The drain pipes opening into the gullies afford an easy path for entrance into and exit from the upper storeys of buildings in the case of *Mus rattus* and also of *Mus decumanus*. The drainage and sewage systems afford shelter to *Mus decumanus*.

TABLE XXI.

Section	Area in acres	Population	Density of population per acre	No. of buildings		Occupied building per acre	Average population per building	Plague per mille	Remark
				Occupied	Vacant				
Bhuleshwar	75.79	38,129	503.0	1123	206	14.6	34.0	17.0	Densely populated residential quarter of poorest class of natives; many shops but few godowns.
Dongri	285.47	32,663	114.4	535	279	1.8	61.0	16.4	Godowns, huts, stables, and poor residential buildings predominate. Buildings much overcrowded.
Colaba	428.11	19,085	44.5	468	226	0.9	40.8	16.3	The population includes the military who are counted as living in one building. Colaba village, which closely resembles the native town, is in this section.
Mazagon	638.59	30,872	48.3	1216	435	1.9	25.0	15.6	Workshops and factories and a few mills. A large number of small huts throughout the section.
Fanasvadi	125.23	29,240	233.4	802	135	6.4	36.0	15.2	Large amount of open ground.
Tarwadi	479.68	26,278	54.7	1057	565	2.2	25.0	14.7	A residential area; a certain number of shops but few godowns. Buildings are not lofty.
Mandvi	164.66	38,158	231.6	915	342	5.5	42.0	14.4	Large amount of open ground: mills and stables fairly common.
Dhobi Talao	99.69	36,594	367.0	1126	193	11.3	33.0	14.2	The section of godowns, which are either single buildings or situated on the ground-floors of dwelling houses. Jains predominate in this section.
Kamathipura	66.14	36,484	551.6	1030	238	15.6	35.0	13.1	Essentially a residential area: has a considerable number of shops and stables. Buildings not lofty.
Market	89.11	35,305	396.1	902	191	10.1	39.0	12.6	Thickly populated district: residential area of poor class. No lofty structures.
Walkeshwar	545.43	12,685	23.2	1354	495	2.5	9.0	12.3	Many native shops and godowns on ground-floors of buildings. Buildings are lofty and closely packed together.
Khara Talao	41.64	26,935	646.8	575	105	13.8	47.0	12.1	Residential area of the wealthiest class: much open ground. Stables in connection with bungalows.
Byculla	511.52	76,280	149.9	1621	501	3.1	47.0	12.0	A thickly populated residential area of poorest class.
Chowpatii	111.77	13,033	116.6	775	306	6.8	17.0	11.7	Considerable number of shops of poor type.
Worli	1,815.64	69,488	38.2	3075	1294	1.6	22.0	11.5	A large scattered district, mills and factories: shops, huts and stables throughout.
									Residential area of rather better class: contains a few mills, shops and huts.
									Extensive plots of vacant ground; many mills.
									Many huts scattered throughout.



2nd Nagpada	34.0	22,016	647.5	486	58	14.3	44.0	11.2	Second most thickly peopled area of Bombay. Residential quarter for poor Mahomedans. A few godowns, some on the ground-floor of dwellings, area of the poorest class.
Khumbharwada	46.06	32,784	711.7	642	96	13.9	51.0	11.0	The most thickly populated quarter: residential area of the poorest class.
Fort North & South	266.0	32,722	123.0	1253	316	4.7	26.1	10.8	The southern part of this section is occupied by better class offices and shops with dwelling houses above. The houses are much better built than in the native quarter. The north part is a rather better class residential area, with shops and a few godowns on ground-floors. Buildings are mostly lofty.
Chakla	51.58	29,362	569.2	812	165	15.7	36.0	10.8	A section of the native town. Very thickly populated. Buildings are lofty, shops or godowns on ground-floor; dwelling houses above. Bulk of the population are Mahomedans.
Oomarkbadi	105.33	53,610	508.9	1041	162	9.9	51.0	10.7	A Mahomedan residential locality of poor class. Thickly populated.
Parel	552.45	46,960	85.0	1182	552	2.1	39.0	10.6	Extensive plots of vacant land. Many mills and workshops. Huts scattered throughout but some large buildings.
Girgaum	124.6	28,449	228.1	1027	206	8.2	28.0	9.4	Residential area of rather better type.
Sewri	445.73	15,985	35.8	976	261	2.1	15.0	8.6	Extensive plots of vacant ground; a few mills. A great number of huts scattered throughout.
Khetwadi	170.3	33,579	197.1	1255	321	7.3	27.0	8.0	Contains residential area both for poor and for better class. Shops and workshops are common. Stables very common.
Tardeo	228.68	28,193	123.2	593	275	2.6	47.0	7.7	Open ground in one portion—more densely crowded buildings in other portion. A few mills; huts and stables common.
Esplanade	663.77	11,015	16.5	598	135	0.9	18.0	6.6	A section of open spaces and great public buildings. It is in consequence sparsely populated.
Sion	4,261.08	30,515	7.1	2400	969	0.5	12.0	6.0	The least densely peopled area of Bombay. Extensive plots of vacant land. A great many huts scattered throughout.
Mahalakshmi	642.01	24,650	38.3	1684	618	2.6	14.0	5.8	In one portion residential quarter of wealthy class; in other portion buildings are of poor class. Many stables.
Mahim	1,286.23	31,178	24.2	2677	847	2.1	11.0	5.5	Extensive vacant plots. Many huts.
1st Nagpada	29.6	3,335	112.6	67	39	2.2	49.0	3.6	Recently reconstructed by Improvement Trust.

2. *General considerations.*

It cannot be doubted that, when judged according to European standards of sanitation, gullies must be regarded as being highly insanitary structures. Nevertheless, excluding the epizootics from consideration, we have not been able to discover any definite relation connecting this state of affairs with the spread of epidemic plague.

We may, for example, contrast the city of Bombay with the villages in the northern portion of the island, namely, Worli, Wadhala and Sion, in which there are no attempts at a conservancy system, the inhabitants using for the purposes of nature the fields beyond the limits of the village. It is a well recognised fact that, when plague has been epidemic in these villages, the proportion of the population affected has been as great, if not greater, than in the case of Bombay City. Again, in the city we have found plague cases in buildings provided with an efficient drainage system. Mention has already been made of a severe epidemic which took place in 1906 in a number of new chawls in Morland Road. These chawls are provided with a modern water carriage system. The privies are outside the houses and are flushed by means of an ample supply of water, the drains being efficiently trapped.

## IV. OVERCROWDING OF THE POPULATION.

It is certain that in some districts of Bombay there is dense overcrowding. Not only are the buildings densely packed together, but in many buildings the cubic space available in the several rooms is small in proportion to the number of inhabitants.

*The question of the relation of overcrowding to the spread of plague.*

Using the same method as that employed by Mr Hankin and cited by the Indian Plague Commission, we have compared the incidence of plague in the different sections with the density of population of each section. The criteria which we have used as an indication of density of population are :

- (a) The number of inhabitants per acre ;
- (b) The number of inhabited buildings per acre ; and
- (c) The average number of inhabitants per occupied building.

As pointed out by the Indian Plague Commission none of these criteria, taken separately, can be considered as an accurate measure of

overcrowding. We need not repeat the obvious objections which were raised in their report to these methods of estimating the density of the population, but shall content ourselves with drawing attention to the data obtained, since we are able to give the assurance, based upon an intimate knowledge of Bombay, that the statistics presented do, as a matter of fact, provide a rough indication of the density of the population in the different sections.

We have constructed a table (XXI.) showing the plague mortality per mille in the different sections during the year September 1905—October 1906, and showing, at the same time, the figures relating to the three criteria of density mentioned above. This shows at once that there is no relation between the severity of plague in these sections and any of the factors which contribute to overcrowding of the population.

Additional evidence on the question at issue has been obtained by an analysis of over 4000 plague case cards, on which was recorded the number of square feet per head in the house in which the plague case occurred. The data thus obtained are set forth in the accompanying table (XXII.), from which it is seen that while no doubt there was dense overcrowding in very many instances, still there was a very considerable percentage of houses in which overcrowding could not be said to exist.

Viewing the evidence as a whole we are of opinion that there is no relation between overcrowding and plague incidence.

TABLE XXII.

*Showing percentage of plague cases in different houses classified according to their condition as regards overcrowding.*

	Houses in which the area per head was 10 sq. feet or less	Houses in which the area per head was between 11 and 25 sq. feet	Houses in which the area per head was between 26 to 50 sq. feet	Houses in which the area per head was more than 50 sq. feet	Total
Number of plague cases	430	1648	1433	700	4211
Percentage of total cases	10·2	39·1	34·1	16·6	—

## V. THE HABITS AND CUSTOMS OF THE PEOPLE.

Closely bound up with the social condition of the people are their habits, which we now pass on to consider.

As we have already pointed out the great majority of the inhabitants of Bombay are poor and live in houses consisting of one room only. In this room the food is cooked and eaten. The people take their meals on plantain leaves or brass platters, placed on the floor, no tables nor chairs being used.

The supplies of raw material—grain, seeds used in the preparation of curry powder, etc.—are stored in wooden chests. The room is also used as a sleeping room, the floor, either bare or with a grass mat, often serving as a bed. Furniture, properly so called, is scanty or may be even absent, but one will often observe in the rooms wooden boxes containing grain, heaps of firewood or of cowdung cakes, used as fuel, brass and earthenware pots, in addition to articles which appear to the European to be useless accumulations of rubbish, but on which the native of India sets considerable store (Plate XXVII). In order to provide storage room for these articles a small loft is often improvised with planks or bamboos either inside the room or on the verandah (Plate XXIII). On this loft is stored all kinds of rubbish and odds and ends, such as earthenware and brass jars, pieces of matting, firewood, old clothes, etc. While the floor of the room is often swept and kept clean, being covered at short intervals with a fresh layer of cowdung, this miscellaneous property is seldom disturbed.

While the living rooms of the people are kept fairly clean this cannot be said of the environs of the buildings. We have already referred to the use the people make of the gullies as depositories for the house refuse. Another native custom, which increases the difficulty of keeping the surroundings of buildings clean, is that of tethering cattle, sheep and goats in the courts and lanes and even in the entrances of buildings.

This description of the habits of the people applies only to the poorer classes. One observes that, as they rise in the social scale, the mode of living becomes more like that of Europeans. The compounds of the houses are kept clean and the household rubbish which accumulates is relegated to outhouses.

*The habits of the people in relation to rat infestation.*

We have already pointed out the relation of the common structural defects in the houses occupied by the poorer classes to the infestation of buildings by rats. Important as these facts are in this connection the habits of the people themselves do much to promote rat infestation of their houses, and may alone constitute a source of grave danger, even when the people occupy well-constructed buildings.

In the first place, the natives manifest a universal indifference to the presence of rats (*Mus rattus*, especially) in their houses, and in some instances, as we have already said, go so far as to protect them



756



Bombay City: interior of a room in a chawl.





from molestation. In the second place, a plentiful supply of food is provided for them both outside and inside the buildings. The refuse thrown into the gullies and streets, the remains of food supplied to the animals tethered in or near the buildings, the grain and other eatable materials stored inside the living rooms, all these things attract and are able to support a very large rat population.

Again, the conditions inside the houses, the boxes left undisturbed for long periods, the accumulations of rubbish and the improvised lofts, offer excellent shelter for *Mus rattus*. It has been with us a common experience when searching the houses and moving the furnishings about for this purpose to come across rats of this species and to find nests of young rats.

As an illustration of the influence of the habits of the people and the conditions resulting therefrom on the spread of plague, we may cite with advantage the case of the chawls in Morland Road, to which passing reference has twice before been made.

These chawls, 16 in number, were built recently by the City Improvement Trust and, in fact, some of them had only just been occupied before the plague epidemic of 1906 began. The walls are solidly built of brick and are supported on a high masonry plinth (Plate XXIV). The floors are of concrete or of patent stone and the roofs of Mangalore tiles. The verandahs and corridors are also paved with concrete and are wide and airy, the lighting and ventilation of the whole building leaving nothing to be desired. There are no gullies, most excellent water-closets being provided.

The buildings in themselves offer no shelter to rats. In spite of this *Mus rattus* is common in the houses. It is certain that the rat infestation of these buildings is due entirely to the habits of the people in the matter of the disposal of their household belongings as described above. Unfortunately for the inhabitants of these chawls this state of things, for which they themselves were largely responsible, had disastrous consequences, for in the epidemic of 1906 the chawls were so badly infected, that the people had to vacate them and live in huts made of bamboos and matting built on an adjoining piece of vacant ground.

These chawls have a population of about 4000 and no fewer than 57 cases of plague occurred amongst the inhabitants. At the time of the epidemic there was a considerable mortality amongst the rats in the chawls and several were proved on examination by us to be plague infected. In some of the rooms in which dead rats had been found and plague cases had occurred a very large number of rat fleas were obtained,

in one instance 263 in a room, on guinea-pigs used as traps and placed in the rooms only after they had been "disinfected." (*Vide* these Reports Vol. VI. Table II. p. 482.) In two instances guinea-pigs used for this purpose died of plague.

#### VI. THE SOCIAL CONDITION OF THE POPULATION.

The general experience of plague workers in Bombay has been that the incidence of the disease is not so great amongst the well-to-do classes as amongst the poorer population. Our own experience bears this out, and, moreover, we have collected certain statistical data which are in harmony with it.

It was considered that the social condition of the people might be indicated by classifying them according to the number of rooms in the house which they occupied. As a matter of fact, we are convinced from personal observation that for statistical purposes this criterion of social condition can be accepted as being approximately exact for Bombay. We have accordingly worked out the relative incidence of plague during the epidemic of 1906 on people who lived in houses of one room, two rooms, three rooms etc. In the accompanying table (XXIII.) are set forth the figures for the whole of Bombay. The figures referring to the population are taken from the census of 1901, the last available. A study of this table shows conclusively that the incidence of plague is greatest on the people inhabiting the smaller houses—one-roomed houses especially—and that the incidence becomes less as the number of rooms in the house becomes greater. We obtained confirmatory evidence of this from similar tables relating separately to 21 of the sections of the City.

It would appear then that the incidence of plague is greater on the poorer than on the well-to-do classes. When it is remembered that the poorer classes constitute the mass of the population—85 % of the total population living in one-roomed houses—it becomes necessary to consider why this should be so. Everything that has been written in this account applies to the poorer class of the population, namely, the buildings in which they live, their manner of living and, most important of all, the danger to which they are exposed from rats. It seems to us, then, that the explanation is to be found in the conditions in which they live and in their habits.

TABLE XXIII.

*Incidence of plague on persons living in houses of 1 Room, 2 Rooms, 3 Rooms, etc.*

	1 Room		2 Rooms		3 Rooms		4 Rooms		5 Rooms		Total
	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	
Bombay Population	581,070	85.1	52,585	7.7	23,093	3.4	16,601	2.4	9,458	1.4	682,807
Plague cases	8,889	92.4	543	5.6	108	1.1	58	0.6	15	0.2	9,613

## VII. SUMMARY.

From the considerations stated above we feel justified in coming to the conclusion that the insanitary conditions which exist in Bombay have no influence—at least none which acts directly—on the spread of epidemic plague.

While this is so, certain almost universal sanitary defects, notably the gully system and the construction of buildings, undoubtedly facilitate the diffusion of epizootic plague throughout the City, and thus indirectly influence the spread of the epidemic.

Even when the people live in well-constructed buildings free from sanitary defects and offering in themselves no shelter to rats, they still remain exposed to danger from rats in their houses. *Mus rattus* is attracted into such houses by the shelter afforded by the little disturbed property of the people, who themselves are quite indifferent to the presence of rats in their houses.



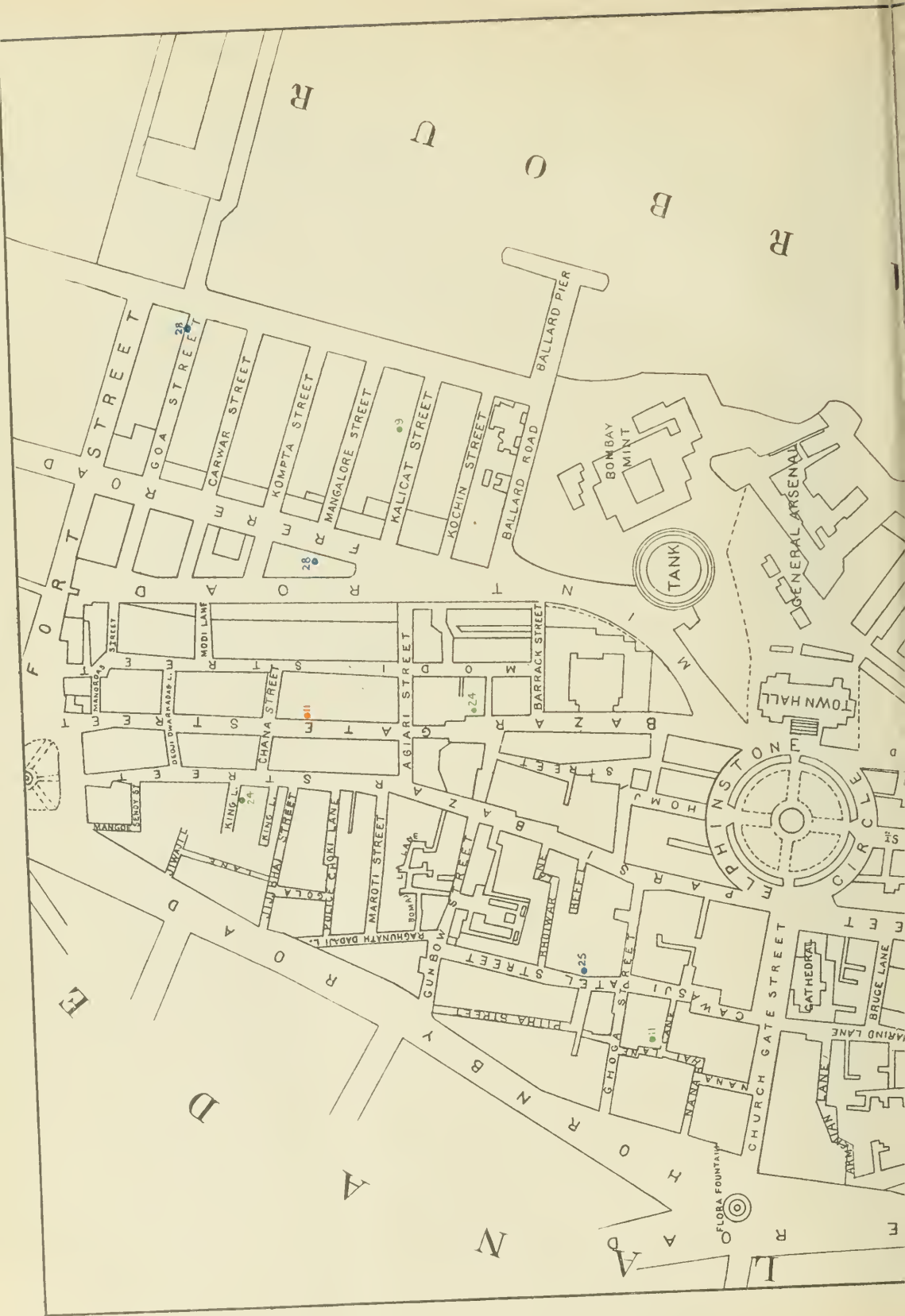
The southern part of the section Fort North and South is occupied by better class offices and shops with dwelling houses above. The houses are much better built than in the northern quarter, which is a native residential area, with shops and a few godowns on the ground-floors. Buildings are mostly lofty.



MAP 2

BOMBAY CITY  
FORT NORTH AND SOUTH

October, 1905





# FORT NORTH AND SOUTH

October, 1905

400 feet

- Plague case
- M. rattus
- M. decumanus





MAP 3

BOMBAY CITY  
FORT NORTH AND SOUTH

November, 1905





# FORT NORTH AND SOUTH

November, 1905

400 feet

Plague case

M. rattus

M. decumanus





MAP 4

BOMBAY CITY  
FORT NORTH AND SOUTH

December, 1905





# FORT NORTH AND SOUTH

December, 1905

400 feet

- Plague case
- M. rattus
- M. decumanus



MAP 5

BOMBAY CITY  
FORT NORTH AND SOUTH

January, 1906







# FORT NORTH AND SOUTH

January, 1906

100 feet

- Plague case
- M. rattus
- M. decumanus



MAP 6

BOMBAY CITY  
FORT NORTH AND SOUTH

February, 1906





FORT NORTH AND  
SOUTH

February, 1906

400 feet

- Plague case
- *M. rattus*
- *M. decumanus*





MAP 7

BOMBAY CITY  
FORT NORTH AND SOUTH

March, 1906



March, 1906

400 feet

- Plague case
- *M. rattus*
- *M. decumanus*







MAP 8

BOMBAY CITY  
FORT NORTH AND SOUTH

April, 1906





# FORT NORTH AND FORT SOUTH

April, 1906

400 feet

- Plague case
- M. rattus
- M. decumanus



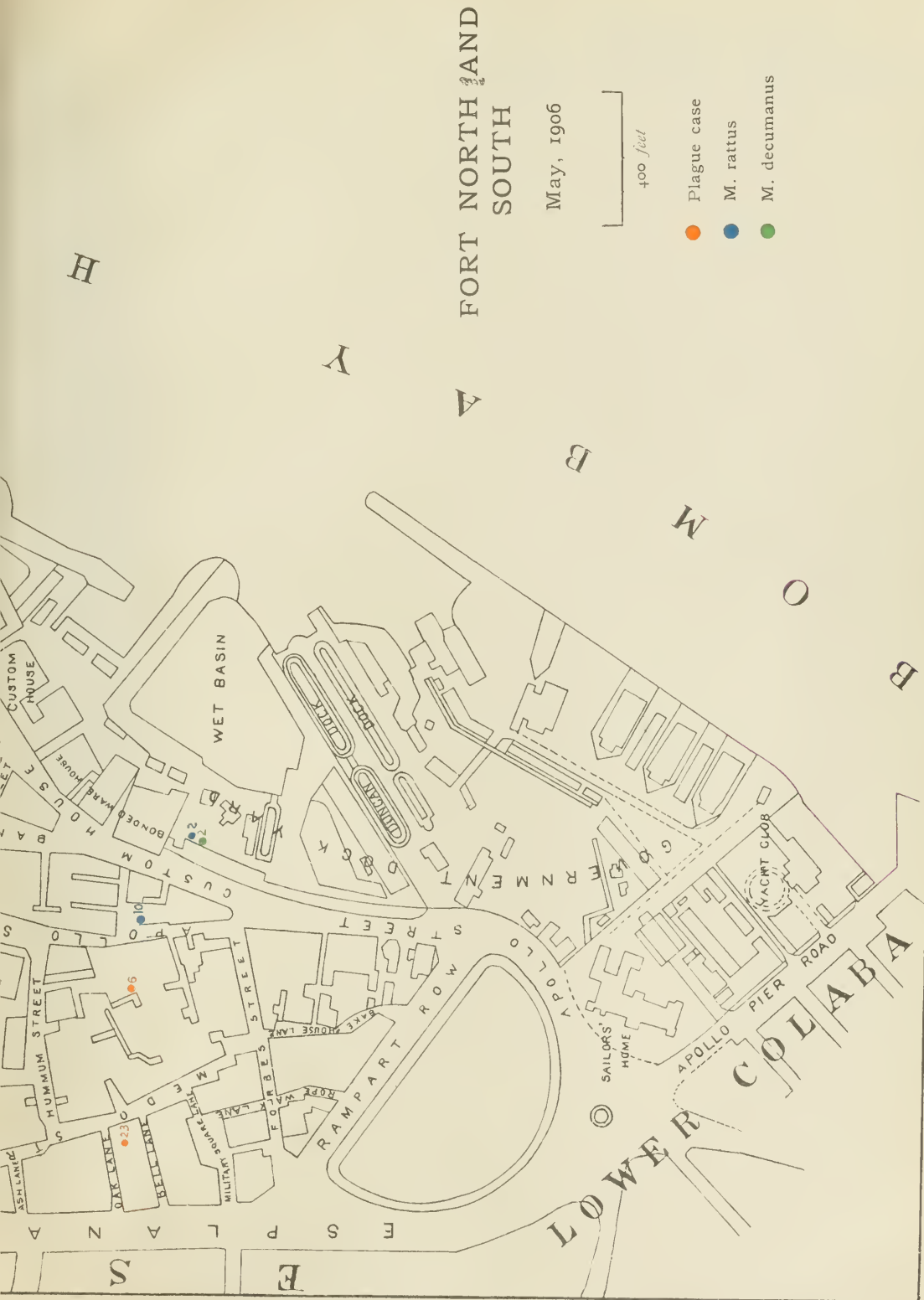


MAP 9

BOMBAY CITY  
FORT NORTH AND SOUTH

May, 1906







MAP 10

BOMBAY CITY  
FORT NORTH AND SOUTH

June, 1906







# FORT NORTH AND FORT SOUTH

June, 1906

100 feet

- Plague case
- M. rattus
- M. decumanus



MAP 11

BOMBAY CITY  
FORT NORTH AND SOUTH

July, 1906







# FORT NORTH AND SOUTH

July, 1906

400 feet

- Plague case
- M. rattus
- M. decumanus



MAP 12

BOMBAY CITY  
FORT NORTH AND SOUTH

August, 1906





# FORT NORTH AND SOUTH

August, 1906

400 feet

- Plague case
- M. rattus
- M. decumanus





MAP 13

BOMBAY CITY  
FORT NORTH AND SOUTH

September, 1906



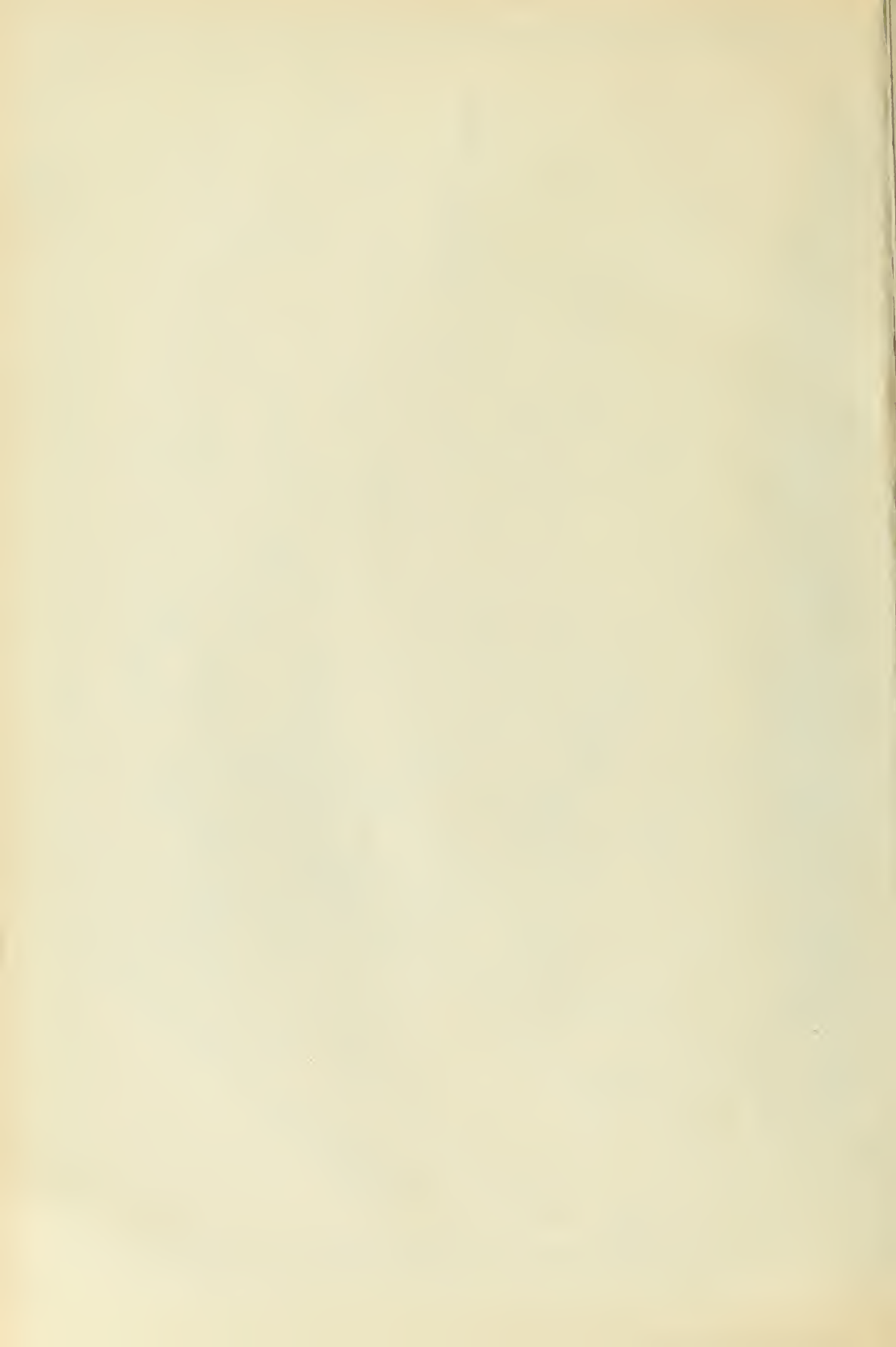


# FORT NORTH AND SOUTH

September, 1906

400 feet

- Plague case
- M. rattus
- M. decumanus





# APPENDIX I.

## Fort North and South.

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
1st to 14th October	3	0	2·3
15th to 28th „	0	2·3	2·3
29th to 11th November	3	0	3·7
12th to 25th „	1	0	2·3
26th to 9th December	7	0	8·2
10th to 23rd „	1	3·5	11·7
24th to 6th January	2	4·2	29·4
7th to 20th „	4	9·4	93·3
21st to 3rd February	6	17·3	113·2
4th to 17th „	19	28·2	164·5
18th to 3rd March	38	25·0	229·8
4th to 17th „	31	32·7	198·3
18th to 31st „	44	51·6	158·9
1st to 14th April	43	46·2	156·8
15th to 28th „	59	57·2	101·5
29th to 12th May	42	39·7	43·2
13th to 26th „	19	17·5	22·2
27th to 9th June	10	6·8	10·7
10th to 23rd „	8	1·2	2·3
24th to 7th July	5	1·4	2·3
8th to 21st „	1	0	4·7
22nd to 4th August	5	1·2	1·4
5th to 18th „	0	4·2	0
19th to 1st September	0	0	0
2nd to 15th „	2	0	5·8
16th to 29th „	0	0	1·4
	13	13·0	52·0

## Mandvi.

1st to 14th October	3	1·2	4·6
15th to 28th „	3	1·2	7·0
29th to 11th November	2	2·6	16·8
12th to 25th „	2	3·5	23·3
26th to 9th December	8	9·3	40·8
10th to 23rd „	3	1·2	45·5
24th to 6th January	3	12·6	68·6
7th to 20th „	5	52·5	194·9
21st to 3rd February	11	72·8	245·2
4th to 17th „	15	135·1	266·6

*Plague in Districts of Bombay*

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
18th to 3rd March	30	132.1	271.8
4th to 17th „	67	205.9	209.4
18th to 31st „	61	116.7	188.5
1st to 14th April	79	100.8	133.0
15th to 28th „	118	166.9	171.5
29th to 12th May	57	105.0	90.6
13th to 26th „	26	54.8	50.2
27th to 9th June	6	30.1	32.4
10th to 23rd „	9	12.8	30.3
24th to 7th July	8	16.8	16.6
8th to 21st „	0	11.7	7.0
22nd to 4th August	6	7.9	13.3
5th to 18th „	6	11.2	36.4
19th to 1st September	7	31.3	29.9
2nd to 15th „	8	31.5	45.5
16th to 29th „	8	30.8	49.0
	21	52.0	88.0

*Chakla.*

1st to 14th October	3	1.2	3.5
15th to 28th „	2	2.3	5.8
29th to 11th November	1	0	2.3
12th to 25th „	1	0	3.5
26th to 9th December	0	2.4	11.7
10th to 23rd „	1	1.2	12.8
24th to 6th January	0	1.4	22.4
7th to 20th „	1	15.1	81.7
21st to 3rd February	10	19.6	168.7
4th to 17th „	4	52.5	161.7
18th to 3rd March	4	46.9	231.2
4th to 17th „	18	70.5	228.7
18th to 31st „	59	80.5	205.8
1st to 14th April	55	70.0	126.0
15th to 28th „	82	58.0	101.1
29th to 12th May	46	17.5	26.8
13th to 26th „	15	8.1	15.1
27th to 9th June	6	2.8	2.8
10th to 23rd „	4	1.2	7.0
24th to 7th July	1	1.2	2.3
8th to 21st „	2	0	4.7
22nd to 4th August	0	1.4	6.1
5th to 18th „	0	0	7.5
19th to 1st September	1	3.5	4.9
2nd to 15th „	1	1.2	1.7
16th to 29th „	1	1.4	2.8
	12	17.0	55.0

Fortnightly periods	<i>Market.</i>		
	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
1st to 14th October	4	0	1·2
15th to 28th „	0	1·2	2·3
29th to 11th November	0	1·2	0
12th to 25th „	0	1·2	3·5
26th to 9th December	0	1·2	1·2
10th to 23rd „	2	1·2	7·0
24th to 6th January	3	0	14·0
7th to 20th „	2	1·2	25·7
21st to 3rd February	6	1·4	25·2
4th to 17th „	6	8·4	55·4
18th to 3rd March	12	25·7	195·1
4th to 17th „	26	43·2	244·4
18th to 31st „	75	99·6	276·5
1st to 14th April	71	93·8	163·8
15th to 28th „	98	51·3	85·2
29th to 12th May	75	15·2	15·2
13th to 26th „	35	4·7	4·7
27th to 9th June	11	1·2	2·8
10th to 23rd „	4	0	1·2
24th to 7th July	2	1·4	0
8th to 21st „	1	0	0
22nd to 4th August	2	0	0
5th to 18th „	1	1·2	0
19th to 1st September	1	0	0
2nd to 15th „	2	0	0
16th to 29th „	4	0	0
	17	13·0	43·0

*Oomarkhadi.*

1st to 14th October	3	2·3	3·5
15th to 28th „	1	1·2	3·5
29th to 11th November	1	0	3·5
12th to 25th „	1	0	11·6
26th to 9th December	0	0	9·3
10th to 23rd „	0	2·3	14·0
24th to 6th January	5	4·2	72·8
7th to 20th „	2	14·0	122·5
21st to 3rd February	6	45·0	308·0
4th to 17th „	33	83·1	319·7
18th to 3rd March	65	71·9	297·5
4th to 17th „	75	61·2	279·4
18th to 31st „	108	60·4	129·7
1st to 14th April	71	32·2	54·6
15th to 28th „	87	44·4	57·2
29th to 12th May	65	10·5	26·9
13th to 26th „	18	8·2	11·7

*Plague in Districts of Bombay*

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
27th to 9th June	10	7·7	7·7
10th to 23rd „	2	2·4	1·2
24th to 7th July	1	6·3	6·1
8th to 21st „	1	3·5	7·0
22nd to 4th August	1	2·6	5·1
5th to 18th	1	6·1	8·6
19th to 1st September	4	8·6	7·5
2nd to 15th „	6	4·7	7·0
16th to 29th „	5	2·8	5·6
	22	18·0	68·0

*Dongri.*

1st to 14th October	2	4·7	3·5
15th to 28th „	1	0	8·2
29th to 11th November	3	0	1·2
12th to 25th „	0	2·3	3·5
26th to 9th December	1	1·2	4·6
10th to 23rd „	0	0	3·5
24th to 6th January	2	1·4	16·8
7th to 20th „	2	0	29·2
21st to 3rd February	12	6·8	43·6
4th to 17th „	20	30·1	159·4
18th to 3rd March	29	41·3	173·6
4th to 17th „	48	110·2	160·4
18th to 31st „	99	57·4	109·4
1st to 14th April	75	39·2	49·0
15th to 28th „	78	37·3	28·0
29th to 12th May	67	18·7	19·8
13th to 26th „	38	14·0	16·6
27th to 9th June	16	6·3	6·3
10th to 23rd „	13	5·8	12·8
24th to 7th July	1	2·6	1·2
8th to 21st „	12	3·5	4·6
22nd to 4th August	3	2·8	10·0
5th to 18th „	7	2·6	7·7
19th to 1st September	2	1·4	7·5
2nd to 15th „	3	9·3	11·7
16th to 29th „	2	9·8	9·8
	20	15·0	34·0

*Dhobi Talao.*

1st to 14th October	2	0	3·5
15th to 28th „	0	0	0
29th to 11th November	1	1·2	0
12th to 25th	1	2·3	2·4
26th to 9th December	0	0	4·6
10th to 23rd „	0	1·2	4·6
24th to 6th January	1	2·8	25·0
7th to 20th „	5	2·3	73·5

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
21st to 3rd February	7	11·4	142·6
4th to 17th „	20	7·5	259·0
18th to 3rd March	34	37·2	225·2
4th to 17th „	63	45·5	228·0
18th to 31st „	96	39·9	227·5
1st to 14th April	70	37·4	131·6
15th to 28th „	96	26·8	95·6
29th to 12th May	62	10·5	29·2
13th to 26th „	36	10·5	9·4
27th to 9th June	10	2·8	8·6
10th to 23rd „	3	0	1·2
24th to 7th July	1	0	4·9
8th to 21st „	2	0	0
22nd to 4th August	3	0	0
5th to 18th „	0	0	1·2
19th to 1st September	3	0	0
2nd to 15th „	1	0	1·7
16th to 29th „	3	0	0
	<hr/> 20	<hr/> 9·0	<hr/> 56·0

*Bhuleshwar.*

1st to 14th October	4	0	4·6
15th to 28th „	2	1·2	1·2
29th to 11th November	0	2·3	2·3
12th to 25th „	1	0	11·7
26th to 9th December	0	0	19·8
10th to 23rd „	1	2·3	28·0
24th to 6th January	2	7·0	51·8
7th to 20th „	2	15·2	86·3
21st to 3rd February	9	20·8	127·9
4th to 17th „	11	48·1	256·0
18th to 3rd March	34	73·4	350·5
4th to 17th „	85	112·5	365·2
18th to 31st „	133	92·4	258·8
1st to 14th April	104	49·0	114·8
15th to 28th „	104	54·8	66·5
29th to 12th May	73	24·5	25·7
13th to 26th „	44	12·9	9·3
27th to 9th June	20	2·8	1·4
10th to 23rd „	4	1·2	2·3
24th to 7th July	4	0	1·2
8th to 21st „	1	2·4	4·7
22nd to 4th August	1	1·4	4·7
5th to 18th „	1	4·0	4·0
19th to 1st September	2	1·2	2·3
2nd to 15th „	2	1·2	0
16th to 29th „	5	1·4	2·8
	<hr/> 24	<hr/> 20·0	<hr/> 69·0



*Plague in Districts of Bombay**Fanaswadi.*

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
1st to 14th October	2	0	2·3
15th to 28th „	1	0	0
29th to 11th November	0	0	0
12th to 25th „	1	0	1·2
26th to 9th December	0	0	0
10th to 23rd „	0	0	0
24th to 6th January	0	0	4·2
7th to 20th „	1	1·2	8·1
21st to 3rd February	6	1·2	40·7
4th to 17th „	19	11·9	40·7
18th to 3rd March	18	14·7	35·6
4th to 17th „	37	19·2	45·5
18th to 31st „	61	14·9	61·8
1st to 14th April	64	7·0	57·4
15th to 28th „	93	15·2	30·3
29th to 12th May	79	4·7	14·0
13th to 26th „	29	2·4	8·2
27th to 9th June	13	2·6	1·4
10th to 23rd „	8	0	0
24th to 7th July	1	0	0
8th to 21st „	5	0	1·2
22nd to 4th August	2	0	1·2
5th to 18th „	2	0	0
19th to 1st September	1	0	1·2
2nd to 15th „	0	0	0
16th to 29th „	2	0	0
	17	3·0	13·0

*Khara Talao.*

1st to 14th October	0	2·4	4·7
15th to 28th „	0	0	0
29th to 11th November	0	0	6·8
12th to 25th „	1	2·3	16·4
26th to 9th December	0	2·3	9·3
10th to 23rd „	0	1·2	22·1
24th to 6th January	0	8·4	46·2
7th to 20th „	1	12·9	74·6
21st to 3rd February	3	16·8	165·7
4th to 17th „	6	36·9	216·5
18th to 3rd March	28	43·4	190·2
4th to 17th „	25	39·7	123·7
18th to 31st „	59	29·9	78·4
1st to 14th April	53	15·4	51·8
15th to 28th „	60	12·9	37·3

# *Reports on Plague Investigations in India*      793

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fort- night of 14 days	Plague infected <i>decumanus</i> corrected for fort- night of 14 days
29th to 12th May	35	8·2	17·5
13th to 26th „	26	5·9	9·3
27th to 9th June	9	6·1	11·0
10th to 23rd „	4	2·4	5·8
24th to 7th July	6	2·3	12·1
8th to 21st „	1	2·3	4·7
22nd to 4th August	0	3·7	9·1
5th to 18th „	1	4·0	4·0
19th to 1st September	0	1·2	10·3
2nd to 15th „	3	0	7·0
16th to 29th „	5	0	1·4
	<hr/> 12	<hr/> 10·0	<hr/> 43·0

## *Khumbharwada.*

1st to 14th October	3	0	2·4
15th to 28th „	0	0	0
29th to 11th November	1	0	2·3
12th to 25th „	0	0	2·4
26th to 9th December	1	0	8·2
10th to 23rd „	1	1·2	9·4
24th to 6th January	2	1·4	15·4
7th to 20th „	1	2·3	28·0
21st to 3rd February	1	7·0	45·5
4th to 17th „	2	12·4	111·1
18th to 3rd March	20	29·4	180·1
4th to 17th „	30	30·3	197·2
18th to 31st „	64	49·2	156·3
1st to 14th April	75	43·4	130·2
15th to 28th „	74	43·2	91·0
29th to 12th May	54	17·5	26·9
13th to 26th „	26	4·7	8·2
27th to 9th June	5	1·4	2·8
10th to 23rd „	1	1·2	0
24th to 7th July	0	0	9·3
8th to 21st	0	0	1·2
22nd to 4th August	1	0	0
5th to 18th „	0	1·2	2·3
19th to 1st September	0	1·4	1·4
2nd to 15th „	0	1·7	0
16th to 29th „	1	0	1·4
	<hr/> 14	<hr/> 9·0	<hr/> 39·0

*Khetwadi.*

Fortnightly periods	Plague mortality	Plague infected <i>rattus</i> corrected for fortnight of 14 days	Plague infected <i>decumanus</i> corrected for fortnight of 14 days
1st to 14th October	1	0	0
15th to 28th „	0	0	0
29th to 11th November	0	1·4	0
12th to 25th „	0	0	0
26th to 9th December	1	0	0
10th to 23rd „	0	0	0
24th to 6th January	0	0	0
7th to 20th „	0	0	4·7
21st to 3rd February	1	2·6	5·1
4th to 17th „	2	1·2	11·4
18th to 3rd March	10	3·7	23·1
4th to 17th „	14	1·2	25·7
18th to 31st „	53	9·1	56·9
1st to 14th April	55	11·2	51·8
15th to 28th „	58	15·2	22·1
29th to 12th May	39	5·9	14·0
13th to 26th „	19	3·5	1·2
27th to 9th June	3	0	1·4
10th to 23rd „	4	0	0
24th to 7th July	2	0	0
8th to 21st „	3	0	0
22nd to 4th August	0	0	0
5th to 18th „	1	0	0
19th to 1st September	1	0	0
2nd to 15th „	1	0	0
16th to 29th „	2	0	0
	10	2·0	8·0

## APPENDIX II.

## REPORT ON CERTAIN PLAGUE STATISTICS.

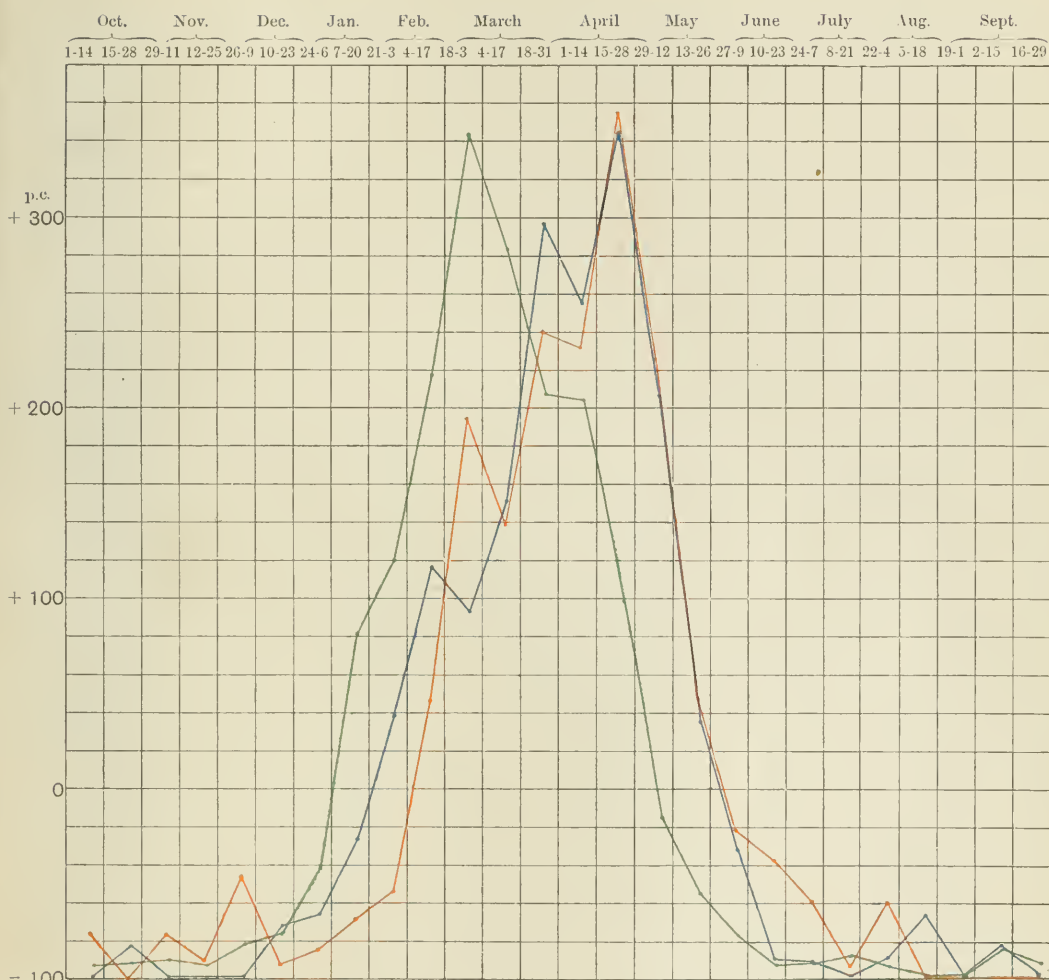
By M. GREENWOOD, JR., M.R.C.S., L.R.C.P.

*(London Hospital Medical School.)*

For the purposes of this investigation I was supplied with the following data :  
 (1) return of all cases of human plague in Bombay for 68 periods of one week :  
 (2) return of total numbers of *M. rattus* brought in and number affected with plague during the same period : (3) the same for *M. decumanus*<sup>1</sup>.

I was requested to determine : (1) what statistical relationship exists between plague in rats and plague in man : (2) whether, supposing such relationship to exist,

<sup>1</sup> The crude figures shown in Table XXIV were used, except that short weeks were corrected to six days.



## FORT NORTH AND SOUTH

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths







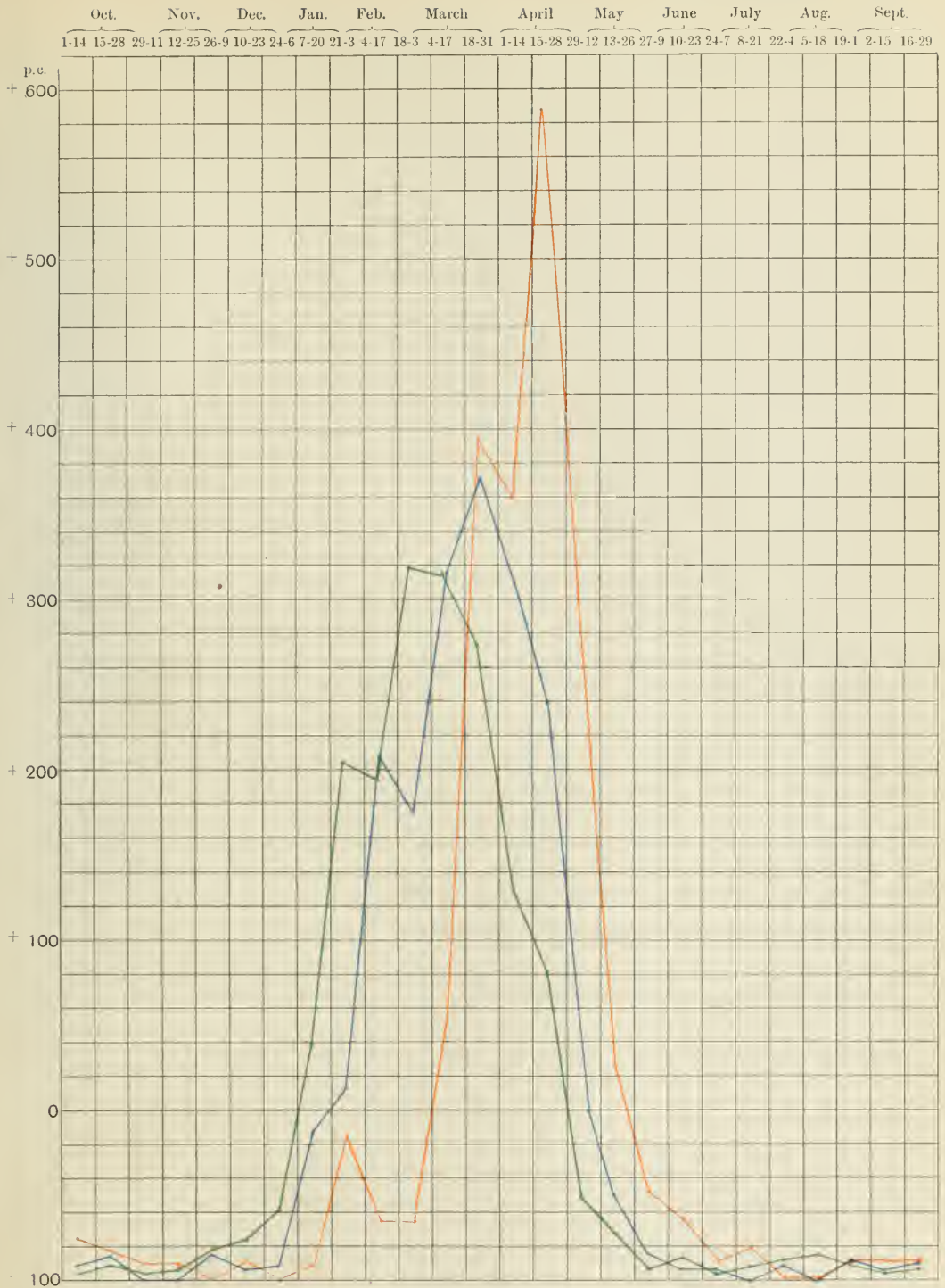
## MANDVI

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths



# BOMBAY VI



## CHAKLA

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths





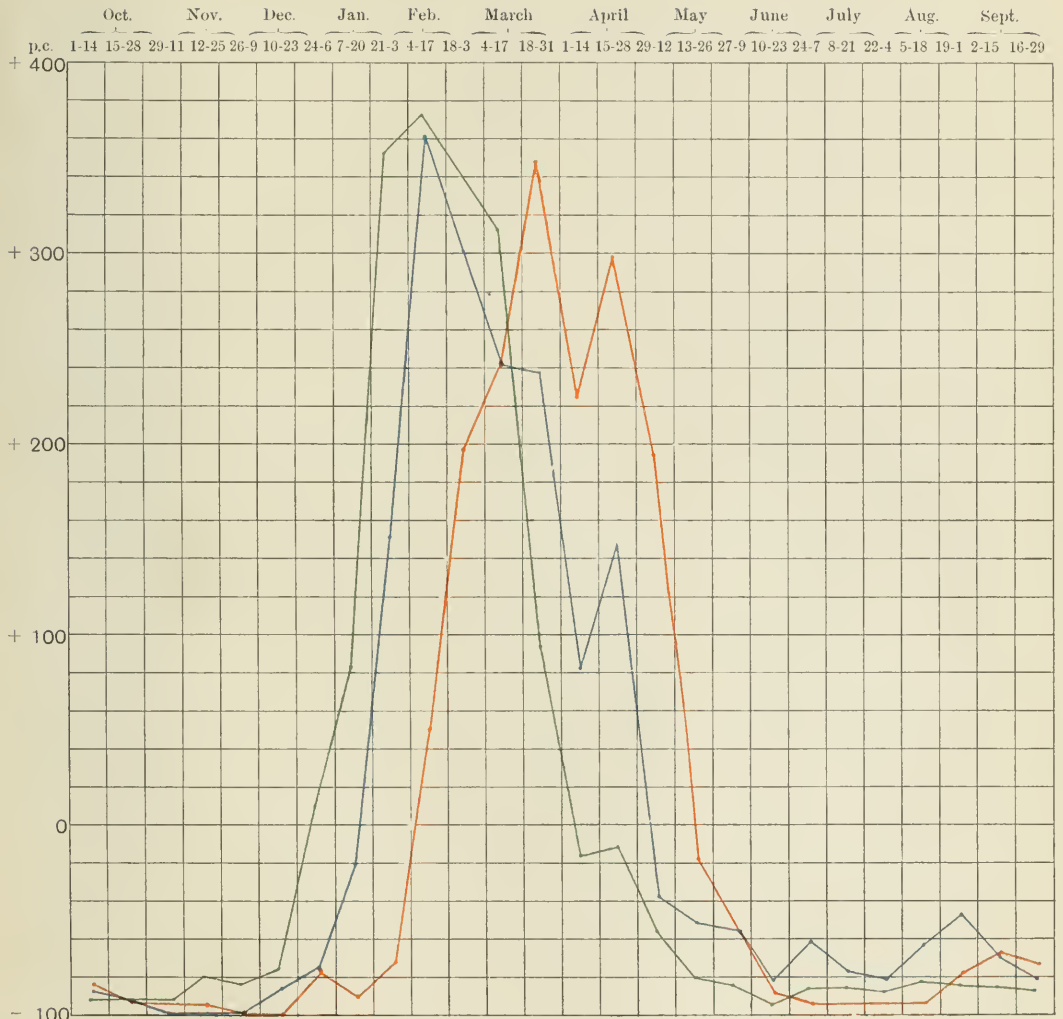
## MARKET

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths







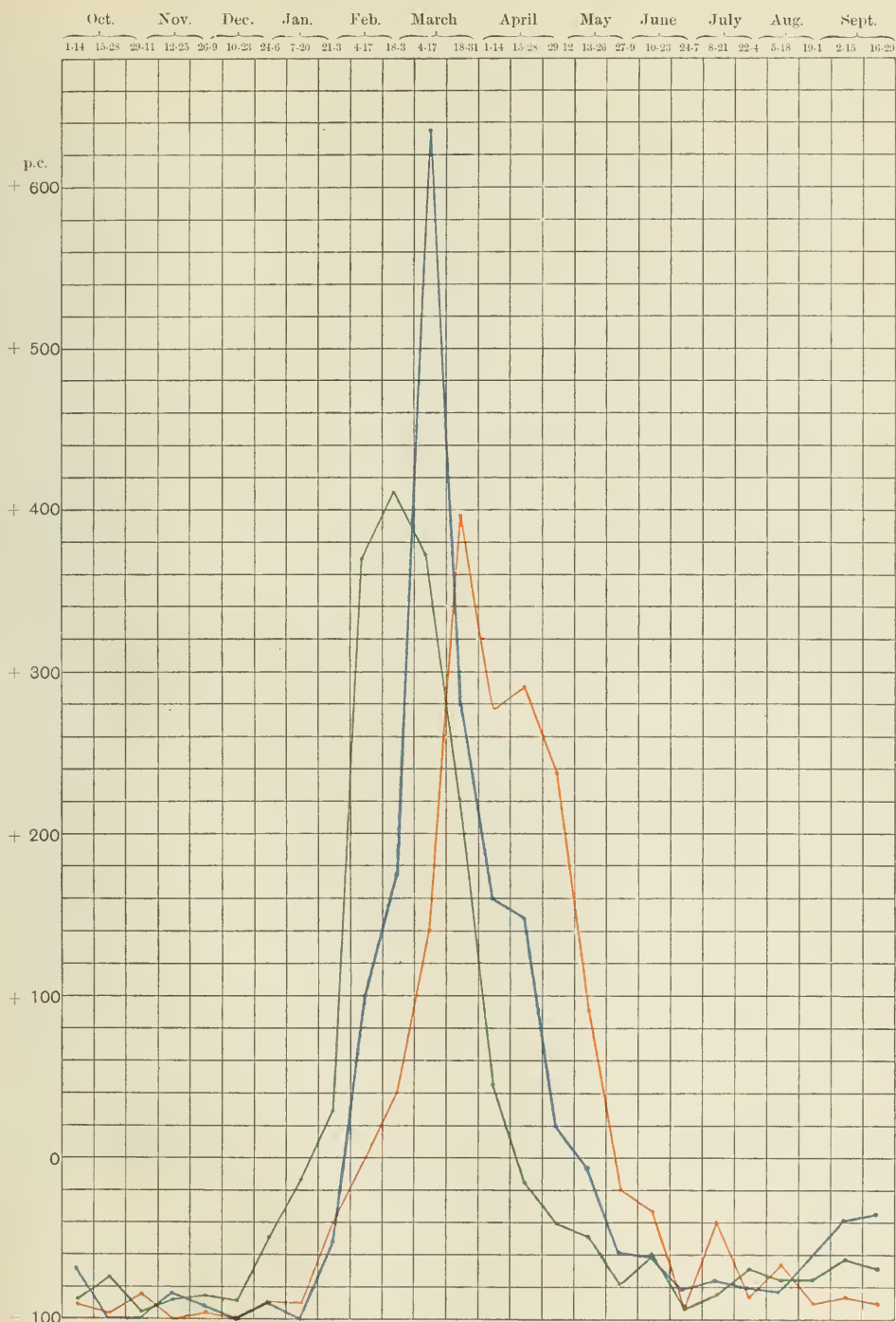
## UMARKHADI

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths



# BOMBAY IX



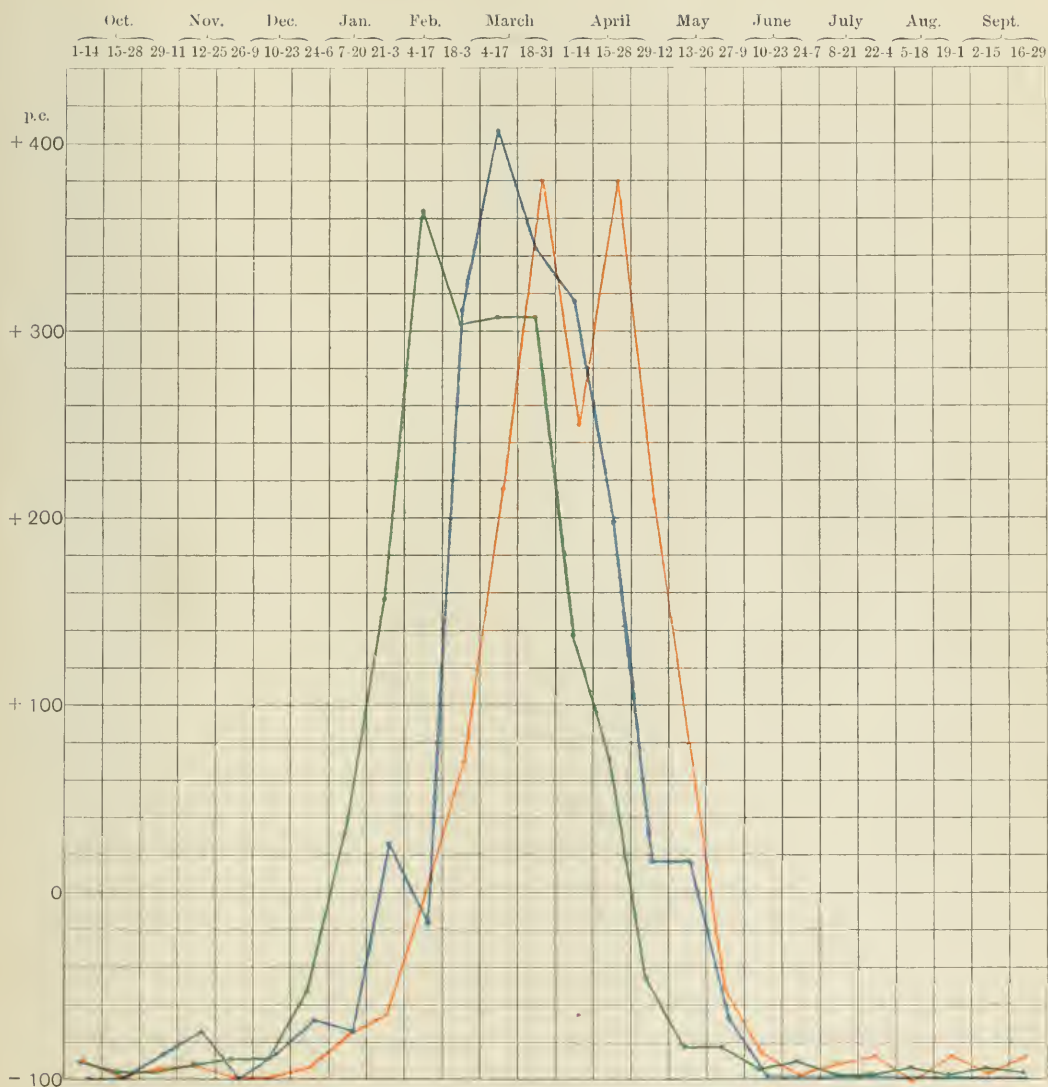
## DONGRI

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths





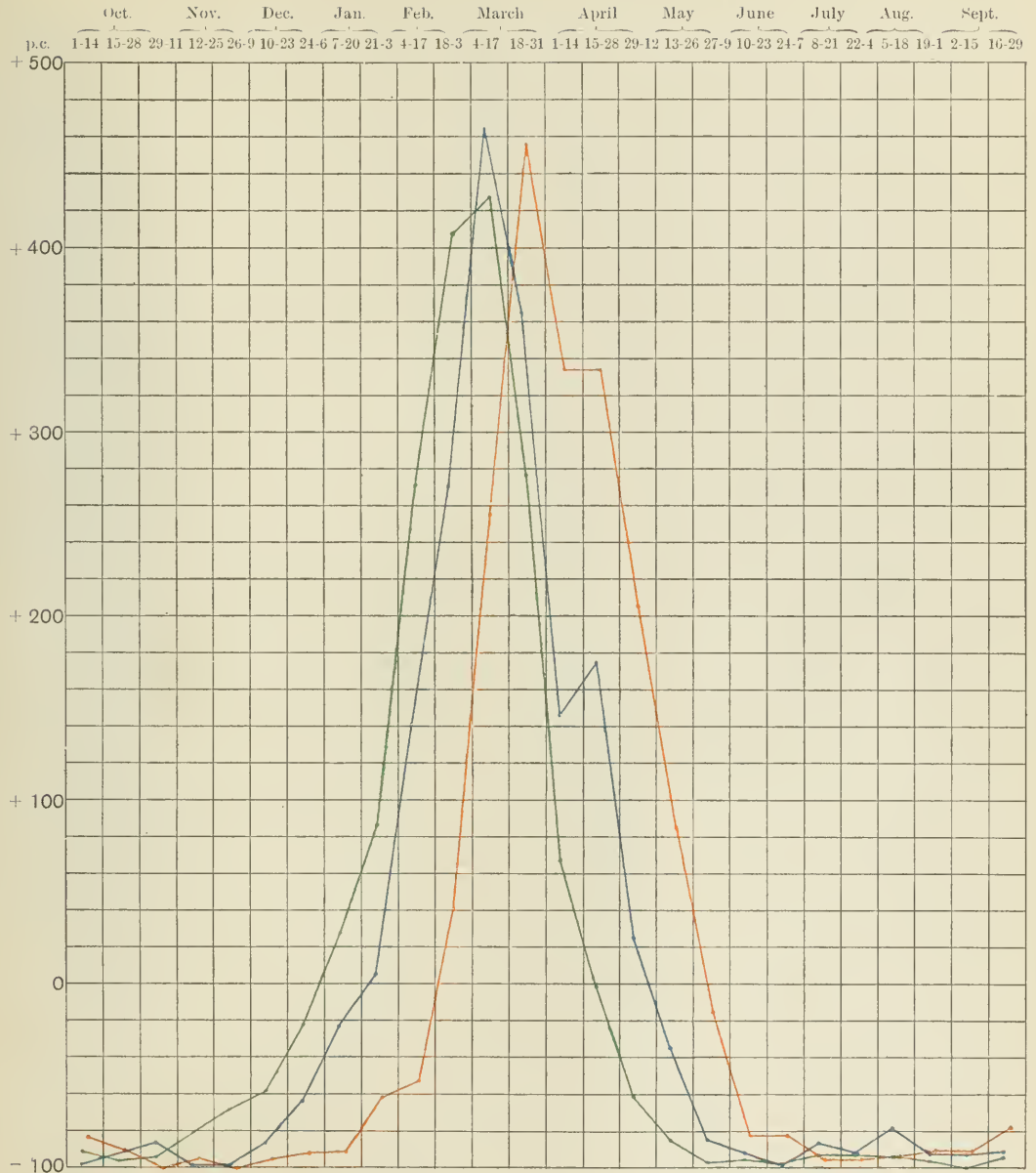


## DHOBI TALAO

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths



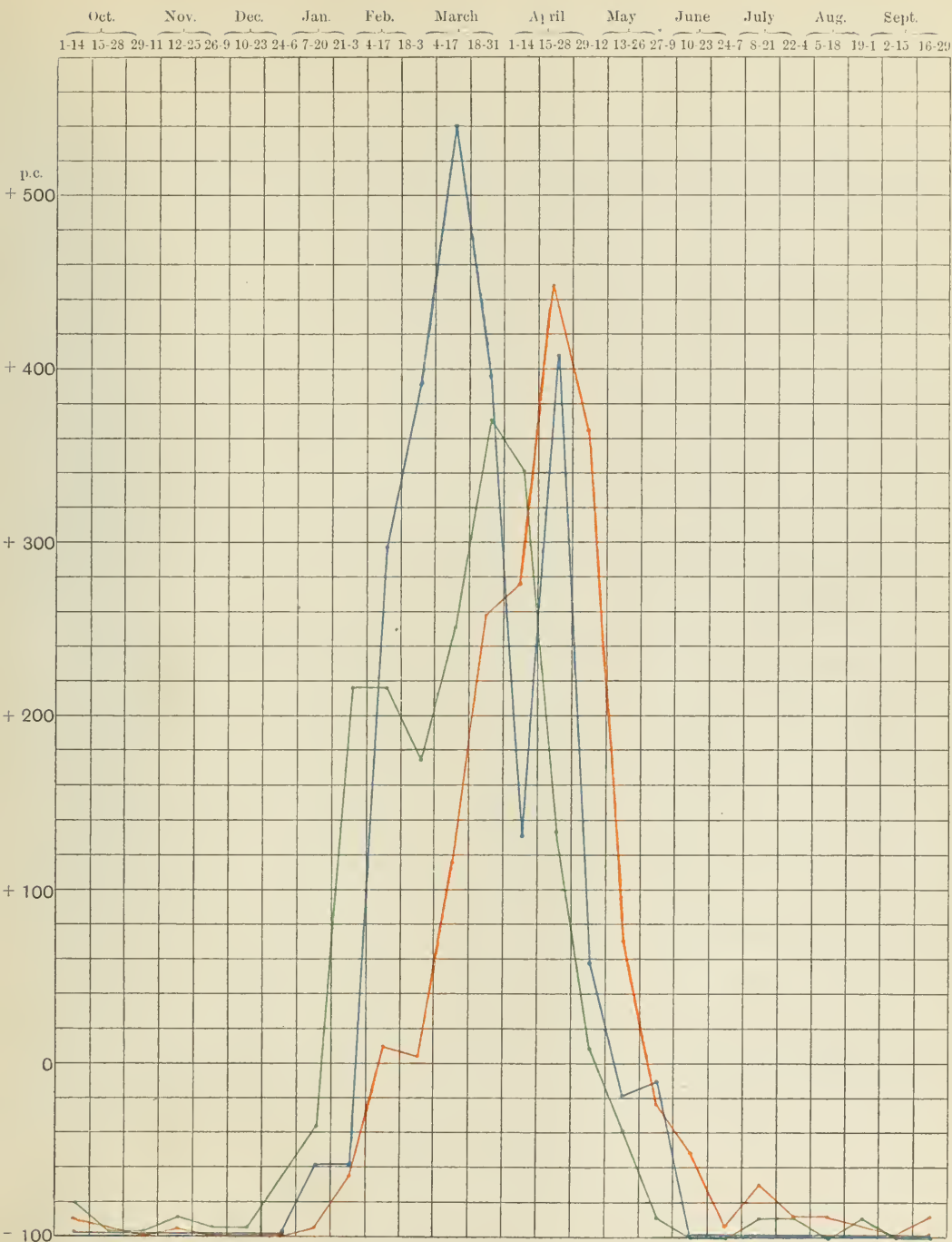


## BULESHWAR

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths





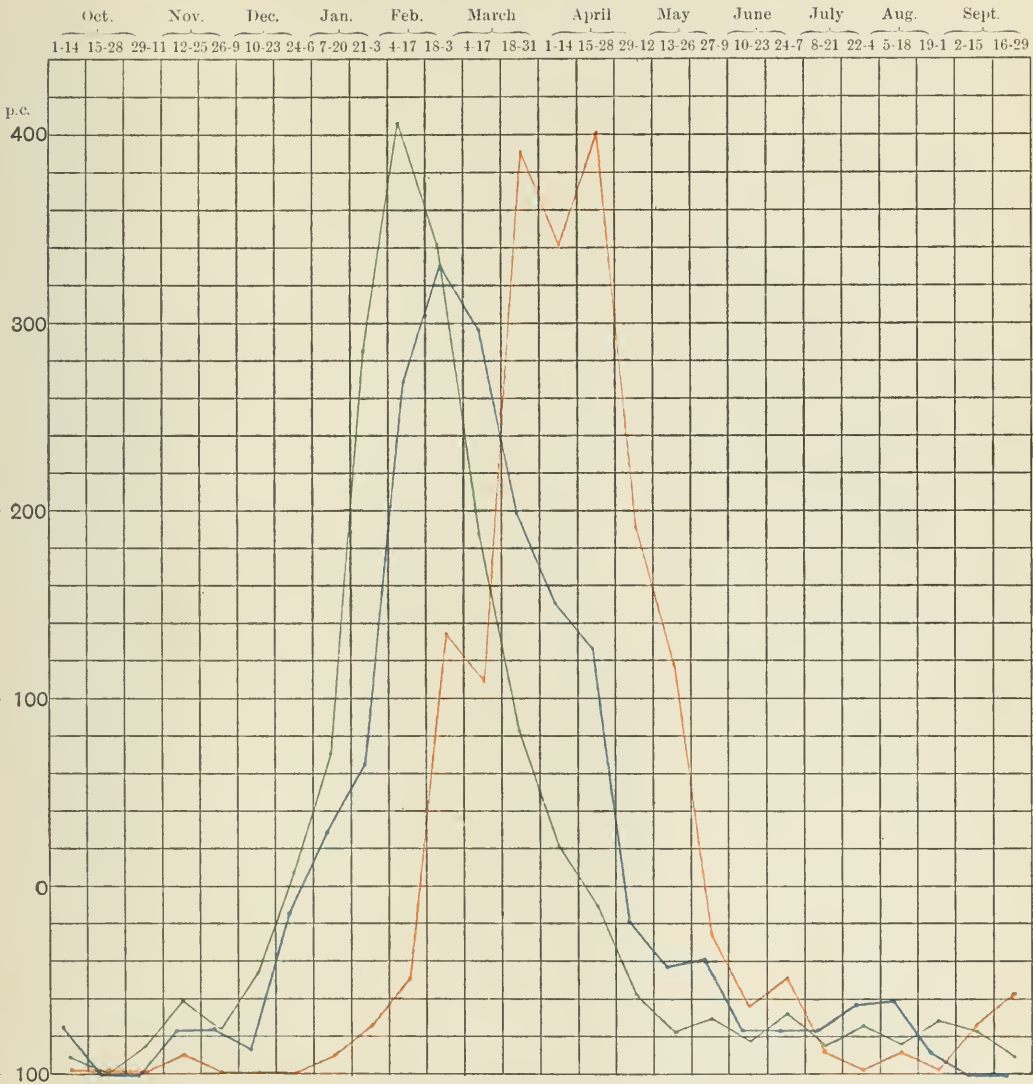
## FANASWADI

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths





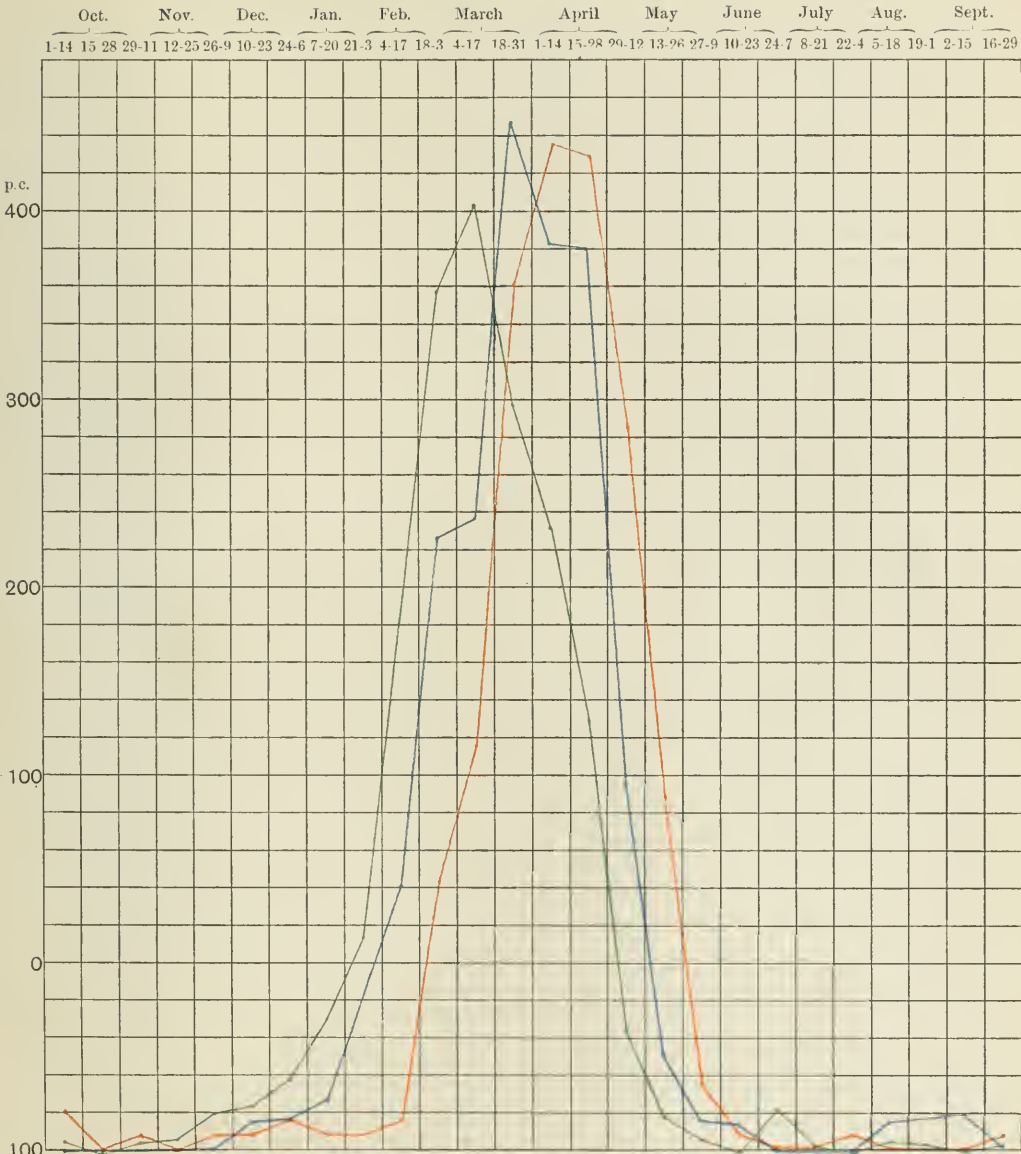


KARA TALAO

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths





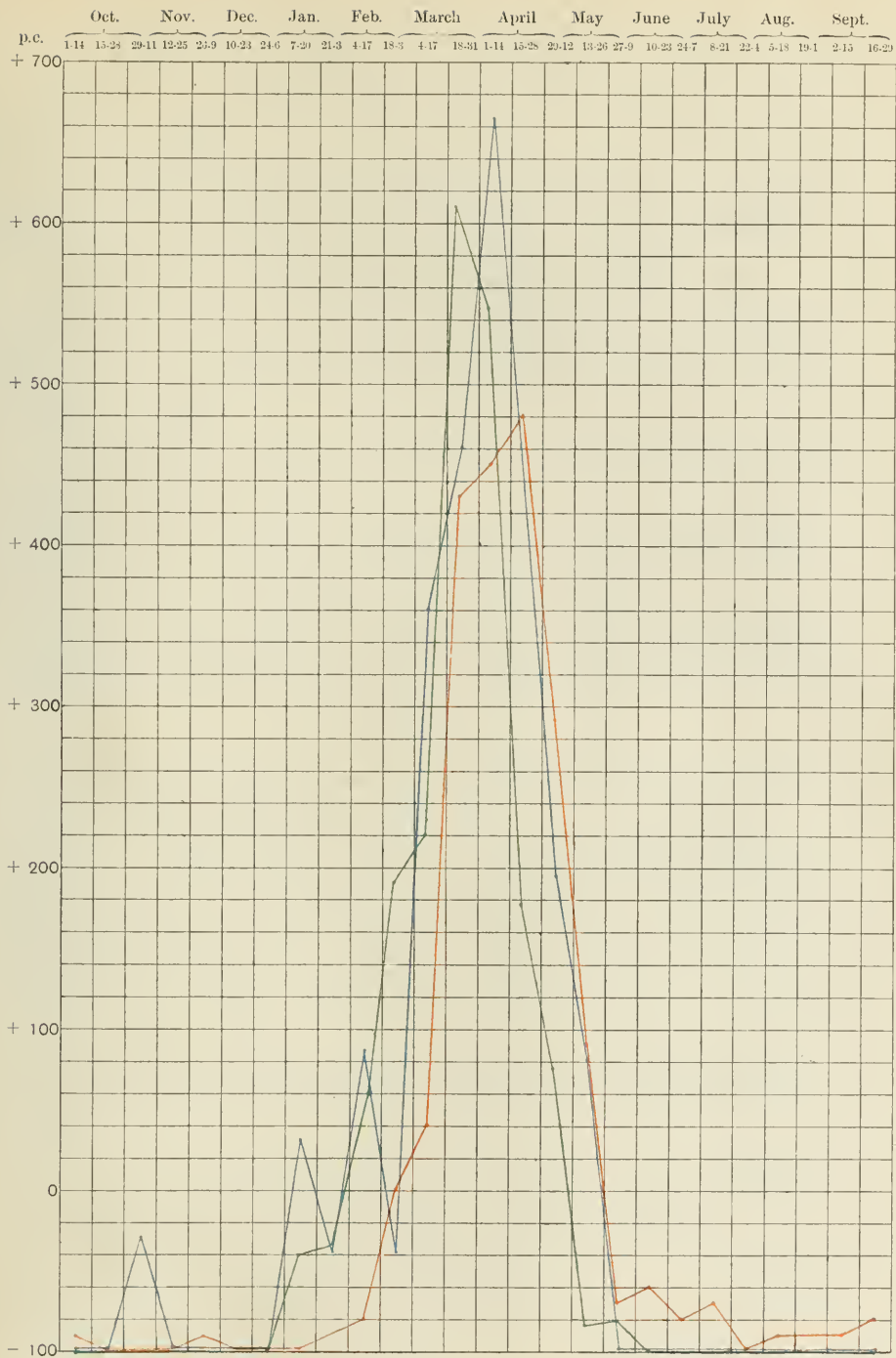
KUMBARWADA

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths







KHETWADI

October, 1905 to September, 1906

- Plague infected decumanus
- Plague infected rattus
- Human plague deaths



the connection is closer between human plague returns for one week and the incidence of rat plague in some earlier week than between the figures for the same period in both cases.

In attempting to answer these questions, two assumptions are necessary: (1) that the diagnosis is certain: (2) that rats are collected uniformly, there being no special activity in bringing them in when plague is rife among human beings. Obviously if the second assumption is unsound, the figures are useless.

I have measured the relationship between human and rat plague as follows.

The coefficients of correlation ( $r$ ) between human plague returns (absolute figures) and rat plague returns (percentages) have been obtained by the usual process. To avoid "grouping errors," each observation was referred separately to the axis. Sheppard's moment corrections were not used, the contact at the ends of the range not being very high.

Taking the figures for the same week, I find the correlation between human plague and plague in *M. rattus* to be  $\cdot8801 \pm \cdot0184^1$ ; between plague in man and plague in *M. decumanus*,  $\cdot7481 \pm \cdot036$ .

At first sight, this appears to suggest almost as close a relationship between *decumanus* and human plague as between the latter and plague in *rattus*. I believe this to be apparent, not real, correlation, for the following reasons.

The correlation between plague in *rattus* and plague in *decumanus* is very high,  $\cdot945 \pm \cdot00875$ , that is, the three variables are related. It is known that, if we have three related variables, the correlation between any two of them for a constant value of the third (the so-called "Partial Correlation Coefficient") is equal to:

$$\frac{r_{12} - r_{13} \cdot r_{23}}{\sqrt{1 - r_{13}^2} \times \sqrt{1 - r_{23}^2}},$$

where  $r_{12}$  is the correlation between the first and second variables,  $r_{23}$  between the second and third,  $r_{13}$  between the first and third.

In the above case, the partial correlation between human plague and plague in *decumanus* is actually negative ( $-\cdot538$ ). If, on the other hand, we calculate the partial correlation between human plague and *rattus* plague for a constant value of *decumanus* plague, we obtain  $\cdot7976$ , which is still very large. In other words, all the correlation between plague in *decumanus* and plague in man is accounted for by the high correlation between plague in *rattus* and plague in *decumanus*; there is no evidence of an independent (positive) connection between human and *decumanus* plague incidence.

If I am right in thinking that *rattus* is more closely associated than *decumanus* with human dwellings, this result is quite intelligible and an argument in favour of the method adopted. For these reasons, I have not further considered the *decumanus* returns.

So far, we have found that the correlation between human and *rattus* returns for the same week is very high; we must next ascertain the values of  $r$  on correlating human plague figures of one week with those of *rattus* for an earlier week.

<sup>1</sup> The non-statistician is reminded that  $r$  must lie between 0 and  $\pm 1$ ,  $\pm 1$  marking (under "normal" conditions) a complete causal relationship.

A negative value simply means that as one variable increases the other decreases, while they both increase together if  $r$  is positive.

Any value of  $r$  greater than  $\cdot75$  is very large.

Showing the crude results of the rat examination.

## M. DECUMANUS

Week ending	Total rats examined	Total examined	Alive	Dead	Plague infected					Per cent.	Per cent. of adult females pregnant	Percent. of young rats
					Total	Per cent.	Alive	Per cent.	Dead			
July 8, 1905	1412	868	385	483	8	0.9	1	0.3	7	1.4	16.0	—
15	1176	692	298	394	9	1.3	1	0.3	8	2.0	13.8	—
22	1137	640	239	401	4	0.6	0		4	1.0	12.2	13.4
27	1330	797	284	513	4	0.5	0		4	0.8	13.7	17.6
Aug. 5	1342	740	245	495	5	0.7	0		5	1.0	11.4	17.3
12	1287	752	255	497	9	1.2	0		9	1.8	9.7	16.0
19	1172	674	219	455	6	0.9	0		6	1.3	12.0	16.0
26	1229	648	236	412	9	1.4	0		9	2.2	11.7	18.5
Sept. 2	1454	805	324	481	8	1.0	1	0.3	7	1.5	16.6	18.8
9	1381	730	252	478	9	1.2	1	0.4	8	1.7	14.2	16.1
16	1808	935	341	594	12	1.3	0	0.0	12	2.0	12.9	22.6
23	1950	954	334	620	19	2.0	2	0.6	17	2.7	14.7	21.6
30	1628	851	386	465	15	1.8	0	0.0	15	3.2	14.3	29.9
Oct. 7	1611	856	418	438	13	1.5	1	0.2	12	2.7	18.4	29.3
14	1654	950	444	506	18	1.9	1	0.2	17	3.4	18.4	27.9
21	1797	1003	413	590	20	2.0	0		20	3.4	14.6	27.0
28	1662	920	336	584	16	1.7	0		16	2.7	16.2	28.3
Nov. 4	1378	902	325	577	23	2.6	0		23	4.0	16.3	28.6
11	1148	712	294	418	15	2.1	1	0.3	14	3.3	13.5	24.9
18	1353	827	276	551	36	4.35	0		36	6.5	14.1	25.6
25	1661	789	242	547	37	4.7	0		37	6.7	17.3	27.6
Dec. 2	2012	1088	378	710	54	5.0	1		53	7.6	16.4	22.3
9	2393	1284	414	870	52	4.0	1	0.2	51	5.9	13.3	22.9
16	2587	1359	471	888	66	4.9	0	0.0	66	7.4	16.1	18.1
23	2345	1136	403	733	83	7.3	3	0.7	80	10.9	13.9	21.3
30	2235	1160	406	754	118	10.2	3	0.7	115	15.2	16.8	21.0
Jan. 6, 1906	2474	1416	449	967	169	11.9	4	0.9	165	17.0	11.1	16.8
13	3369	2106	395	1711	282	13.4	8	2.0	274	16.0	11.8	17.7
20	4320	2891	369	2522	476	16.5	1	0.3	475	18.8	8.9	17.2
27	5510	4131	320	3811	576	13.9	2	0.6	574	15.0	8.5	14.8
Feb. 3	5324	4030	251	3779	610	15.1	2	0.8	608	16.0	9.7	15.3
10	4443	3197	128	3069	715	22.4	3	2.2	712	23.2	12.0	13.8
17	5734	4096	264	3832	1014	24.8	6	2.3	1008	26.3	11.5	15.0
24	4182	2981	119	2862	944	31.7	5	4.2	939	32.8	15.5	12.7
March 3	4705	3295	170	3125	1136	34.5	3	1.8	1133	36.3	18.2	16.1
10	2606	1812	126	1686	725	40.0	8	6.3	717	42.5	17.5	14.0
17	3782	2666	105	2561	1129	42.3	5	4.8	1124	43.9	20.3	16.7
24	3216	2106	89	2017	838	39.8	5	5.6	833	41.3	23.7	17.6
31	3416	2205	116	2089	891	40.4	3	2.6	888	42.5	18.8	17.0
April 7	2596	1600	120	1480	512	32.0	3	2.5	509	34.4	17.7	18.6
14	2688	1671	132	1539	560	33.5	3	2.5	557	36.2	16.3	20.5
21	3284	1869	139	1730	616	33.0	5	3.6	611	35.3	17.3	19.7
28	2350	1391	134	1257	380	27.3	1	0.7	379	30.1	20.5	21.9
May 5	1990	1131	101	1030	214	18.9	2	2.0	212	20.6	19.0	20.2
12	1821	1046	110	936	150	14.3	1	0.9	149	15.9	16.8	20.3
19	1616	932	114	818	111	11.9	1	0.9	110	13.4	15.2	20.3
26	1171	693	121	572	84	12.1	3	2.5	81	14.2	17.6	15.4
June 2	993	557	76	481	54	9.7	2	2.6	52	10.8	16.0	17.4
9	961	510	90	420	29	5.7	0		29	6.9	10.2	15.7
16	1050	589	115	474	33	5.6	0		33	7.0	18.8	13.8
23	1141	564	144	420	31	5.5	0		31	7.4	11.7	18.0
30	1087	507	103	404	22	4.3	1	1.0	21	5.2	13.6	16.2
July 7	1252	558	181	377	28	5.0			28	7.4	18.0	17.2
14	1175	540	128	412	13	2.4			13	3.2	18.6	23.0
21	1261	591	146	445	21	3.6			21	4.7	18.7	16.8
28	1327	620	167	453	29	4.7			29	6.4	16.3	17.9
Aug. 4	1133	469	100	369	19	4.1			19	5.1	12.3	21.5
11	1070	494	128	366	26	5.3			26	7.1	14.9	24.7
18	1411	552	138	414	38	6.9			38	9.2	20.8	20.7
25	1272	554	165	389	24	4.3			24	6.2	20.0	17.5
Sept. 1	1630	722	225	497	38	5.3	2	0.9	36	7.2	15.9	24.7
8	1607	723	168	555	45	6.2			45	8.1	16.2	21.2
15	1031	516	119	397	22	4.3			22	5.5	13.9	18.2
22	1841	822	163	659	34	4.1			34	5.2	11.1	19.8
29	1396	650	136	514	25	3.8			25	4.9	16.3	19.2
Oct. 6	2460	1196	240	956	32	2.7	2	0.8	30	3.1	16.6	26.3
13	3226	1574	404	1170	62	3.9	1	0.25	61	5.2	12.9	29.4
20	1886	808	285	523	27	3.3			27	5.2	12.0	37.9

## M. RATTUS

## HUMAN

747

Total examined	Plague infected								Per cent. of adult females pregnant	Per cent. of young rats	HUMAN		
	Alive	Dead	Total	Per cent.	Alive	Per cent.	Dead	Per cent.			Cases	Deaths	Remarks
544	322	222	10	1.8			10	4.5	22.9	—	53	54	7 days
484	314	170	4	0.8			4	2.3	20.8	—	61	52	
497	315	182	1	0.2			1	0.5	25.7	22.5	45	47	
533	266	267	7	1.3	1	0.4	6	2.2	33.3	22.9	43	39	
602	336	266	5	0.8			5	1.9	22.0	22.8	52	49	
535	299	236	7	1.3			7	2.9	23.3	27.5	73	60	
498	305	193	8	1.6	1	0.3	7	3.6	26.6	23.3	51	53	
581	371	210	9	1.6			9	4.3	23.5	23.8	39	39	5 days only
649	383	266	3	0.5			3	1.1	31.7	24.0	45	38	7 days
651	364	287	4	0.6	1	0.3	3	1.0	17.0	27.1	39	38	
873	529	344	4	0.5			4	1.2	22.9	24.6	37	37	
996	597	399	10	1.0			10	2.5	20.1	27.9	52	37	
777	527	250	4	0.5			4	1.6	23.2	27.2	39	37	
755	500	255	5	0.7			5	2.0	27.9	33.5	32	31	
704	457	247	6	0.8	1	0.2	5	2.0	28.0	29.9	22	19	
794	551	243	9	1.1	2	0.4	7	2.9	27.5	32.9	24	17	
742	484	258	3	0.4			3	1.2	26.0	35.0	9	10	
476	251	225	5	1.0			5	2.2	26.7	33.2	20	14	
436	237	199	1	0.2			1	0.5	23.9	30.0	10	10	5 days only
526	236	290	7	1.3			7	2.4	33.0	41.1	15	12	
872	624	248	4	0.5			4	1.6	20.9	42.3	8	7	
924	617	307	9	1.0	1	0.2	8	2.9	35.2	29.0	12	10	
1109	715	394	11	1.0	1	0.1	10	2.5	18.1	30.4	13	13	
1228	835	393	9	0.7	1	0.1	8	2.0	27.2	41.2	11	8	
1209	804	405	7	0.6	1	0.1	6	1.5	19.2	34.6	12	10	
1075	736	339	13	1.2			13	3.8	23.0	31.3	11	8	5 days only
1058	659	399	18	1.7			18	4.5	21.6	31.1	19	15	5 days only
1263	707	556	32	2.5	1	0.1	31	5.6	22.8	27.7	22	17	
1429	632	797	84	5.9	2	0.3	82	10.3	18.5	24.4	33	28	
1379	561	818	74	5.4	1	0.2	73	8.9	15.5	29.2	60	49	
1294	442	852	107	8.3	0		107	12.6	17.6	24.7	86	71	5 days only
1246	332	914	145	11.6	0		145	15.9	19.6	20.9	136	104	4 days only
1638	442	1196	262	16.0	7	1.6	255	21.3	23.3	20.3	151	134	
1201	275	926	203	16.9	4	1.5	199	21.5	22.7	16.3	220	191	5 days only
1410	353	1057	284	20.1	2	0.6	282	26.7	29.0	20.8	339	303	
794	179	615	219	27.6	3	1.7	216	35.1	22.5	22.3	426	379	4 days only
1116	103	1013	365	32.7	4	3.9	361	35.6	21.3	19.5	541	473	5 days only
1110	212	898	323	29.1			323	35.2	32.0	22.7	793	706	5 days only
1211	174	1037	315	26.0	4	2.3	311	30.0	31.5	20.2	946	836	
996	273	723	223	22.4	2	0.7	221	30.6	36.3	25.1	884	800	5 days only
1017	236	781	243	23.9	4	1.7	239	30.6	28.4	26.1	908	804	5 days only
1415	364	1051	382	27.0	5	1.4	377	35.9	33.6	28.0	1164	1002	
959	170	789	271	28.3	14	8.2	257	32.6	29.3	24.2	1227	1101	
859	245	614	159	18.5	4	1.6	155	25.2	34.0	25.3	1086	971	
775	214	561	142	18.3	2	0.9	140	25.0	36.0	23.4	808	740	
684	202	482	110	16.0	2	1.0	108	22.4	26.0	28.0	624	566	
478	184	294	46	9.6	2	1.1	44	15.0	36.8	31.2	467	416	
436	149	287	43	9.9			43	15.0	24.5	20.9	246	224	5 days only
451	187	264	25	5.5			25	9.5	26.9	15.3	149	133	
461	199	262	11	2.4	1	0.5	10	3.8	20.8	24.7	92	86	
577	270	307	16	2.8			16	5.2	25.5	30.2	57	51	
580	322	258	14	2.4			14	5.4	28.0	24.0	50	44	5 days only
694	405	289	12	1.7			12	4.2	26.3	23.9	44	35	
635	365	270	8	1.3			8	3.0	32.1	22.7	40	38	
670	347	323	13	1.9			13	4.0	31.6	19.1	32	28	
707	403	304	7	1.0			7	2.3	26.0	24.6	42	37	
664	407	257	16	2.4	1	0.25	15	5.8	24.2	30.9	35	35	5 days only
576	335	241	12	2.1			12	5.0	30.9	26.2	35	26	5 days only
859	513	346	19	2.2	1	0.2	18	5.2	34.2	29.1	56	48	
718	385	333	14	1.9			14	4.2	35.2	25.2	31	26	5 days only
908	505	403	27	3.0			27	6.7	30.3	27.8	23	12	
884	506	378	16	1.8			16	4.2	31.1	25.2	29	30	
515	224	291	21	4.1			21	7.2	33.3	24.7	24	29	4 days only
1019	619	400	22	2.2			22	5.5	33.8	29.1	19	30	5 days only
746	344	402	15	2.0			15	3.7	23.3	26.3	39	44	5 days only
1264	614	650	27	2.1	1	0.2	26	4.0	27.5	30.9	28	41	
1652	877	775	38	2.3	5	0.6	33	4.0	27.6	31.1	18	25	
1078	644	434	15	1.4			15	3.5	28.0	35.0	26	32	4 days only



I obtained the following results :—

- (1) Human plague with *rattus* plague in previous week.  $r = .9305 \pm .011$ .
- (2) Human plague with *rattus* plague in 2nd previous week.  $r = .9407 \pm .0096$ .
- (3) Human plague with *rattus* plague, 3rd previous week.  $r = .9206 \pm .0128$ .
- (4) Human plague with *rattus* plague, 4th previous week.  $r = .887 \pm .018$ .

The difference between (1) and (2) is  $.0102 \pm .0146$ ; between (2) and (3)  $.0201 \pm .016$ ; between (3) and (4)  $.0336 \pm .0221$ .

The differences between the correlation coefficient for the same week and (1), (2), (3) and (4) are  $.0504 \pm .021$ ;  $.0606 \pm .021$ ;  $.0405 \pm .0224$ ;  $.0069 \pm .0257$ , respectively.

A comparison of these differences with their "probable errors" makes it clear that with one doubtful exception, there is no real increase in the correlation when we go back in this way. In the case of (2)  $r$  differs from the value previously obtained by nearly three times the "probable error" of the difference; that is to say, there is some evidence that human plague incidence in any given week is most closely associated with *rattus* plague incidence in the next week but one before. But if we adopt as a criterion of significance an increase of three times the "probable error," and most biometricians consider this best, the difference is within the limit. The evidence on this point is therefore merely suggestive, not convincing.

The inferences which may, I think, be drawn from this analysis are :—

(1) There is an extremely close relationship between the incidence of plague in man and plague in *M. rattus*.

(2) There is no undoubtedly significant difference between the results obtained by correlating human plague returns with those of *rattus* for the same and four preceding weeks. One value alone approaches the limit.

(3) The correlation between plague in man and plague in *M. decumanus* is probably spurious, depending on the correlation between plague in *decumanus* and in *rattus*.

## XXIII. OBSERVATIONS MADE IN FOUR VILLAGES IN THE NEIGHBOURHOOD OF BOMBAY.

- I. Introduction.
- II. Observations in Sion.
- III. Observations in Wadhala.
- IV. Observations in Parel.
- V. Observations in Worli.

### I. INTRODUCTION.

We have already drawn attention to the many difficulties which surround an epidemiological study of plague in a city like Bombay and have pointed out that these difficulties might be overcome to a considerable extent if smaller, less scattered, and more isolated communities were put under observation. For this purpose we chose four villages in the neighbourhood of Bombay, namely Sion, Parel, Wadhala and Worli, and two in the Amritsar District of the Punjab, to wit, Kasel and Dhand. In all these villages plague had annually recurred for several years past.

We propose now to describe the course of events in the four Bombay villages.

These villages were selected for the reasons that they occupied isolated positions, and that the inhabitants of at least three of them followed an employment (as fishermen and agriculturists) which kept them confined for the most part to their villages and to the tract of country immediately surrounding them. It was considered, therefore, that the chances of importation of infection from Bombay City were small, thus rendering it less difficult to inquire into the source of infection of each case of plague as it occurred. Moreover, one of the main objects we had in view was to watch carefully for the first cases of rat and human plague and to discover, if possible, their origin and relationship.

As a first step a census of the inhabitants was taken and a detailed examination of each village was subsequently made. For the purpose of this census special "Census Cards" were utilised, on which particulars including the name, age, sex, caste and occupation of every inhabitant were recorded, all these details being entered by one of the members of the Commission.

In order to facilitate reference to events which might happen in connection with a particular house, a system of numbering the houses was adopted. The whole village was partitioned out into convenient divisions, consisting of from 20 to 50 buildings: these divisions we termed 'blocks.' A 'building' we defined as a structure surrounded on all sides by open space and occupied by one or more families. Lastly a 'house,' according to our system of numbering, corresponds to the living room or rooms of a single family. The buildings and houses were numbered in order serially. According to this nomenclature a small hut consisting of a single room and inhabited by a single family is entitled to the appellation of a 'building' as well as of a 'house.' Maps also were prepared, showing each house exactly to scale<sup>1</sup>.

A daily collection of rats was organised; traps were daily set in many of the houses, the buildings being systematically trapped in this way. This part of the scheme was supervised by native clerks, each assisted by two coolies residing in the villages and familiar with their inhabitants. These clerks proved useful in obtaining for us early information regarding plague rats and cases of human plague. Further, the inhabitants were instructed and constantly reminded that all dead rats which were found should be handed over to our clerks. The live rats caught in the traps and any dead rats which were obtained were daily brought to the laboratory and examined in the manner we have already described.

As a means of gaining the confidence of the people a dispensary was opened during the epidemic period, and was placed in charge of a Hospital Assistant, whose duty it was to keep himself acquainted with any occurrences of importance from our point of view.

We sought on more than one occasion to secure the co-operation of the village headmen, but although apparently willing to give us help, it was found by experience that but little assistance was to be looked for from these men or from the villagers themselves. Fortunately no active opposition was met with, but our difficulties in carrying out the scheme

<sup>1</sup> The key map of Sion Village is reproduced below (Map I): the other villages were dealt with in the same way.

MAP I

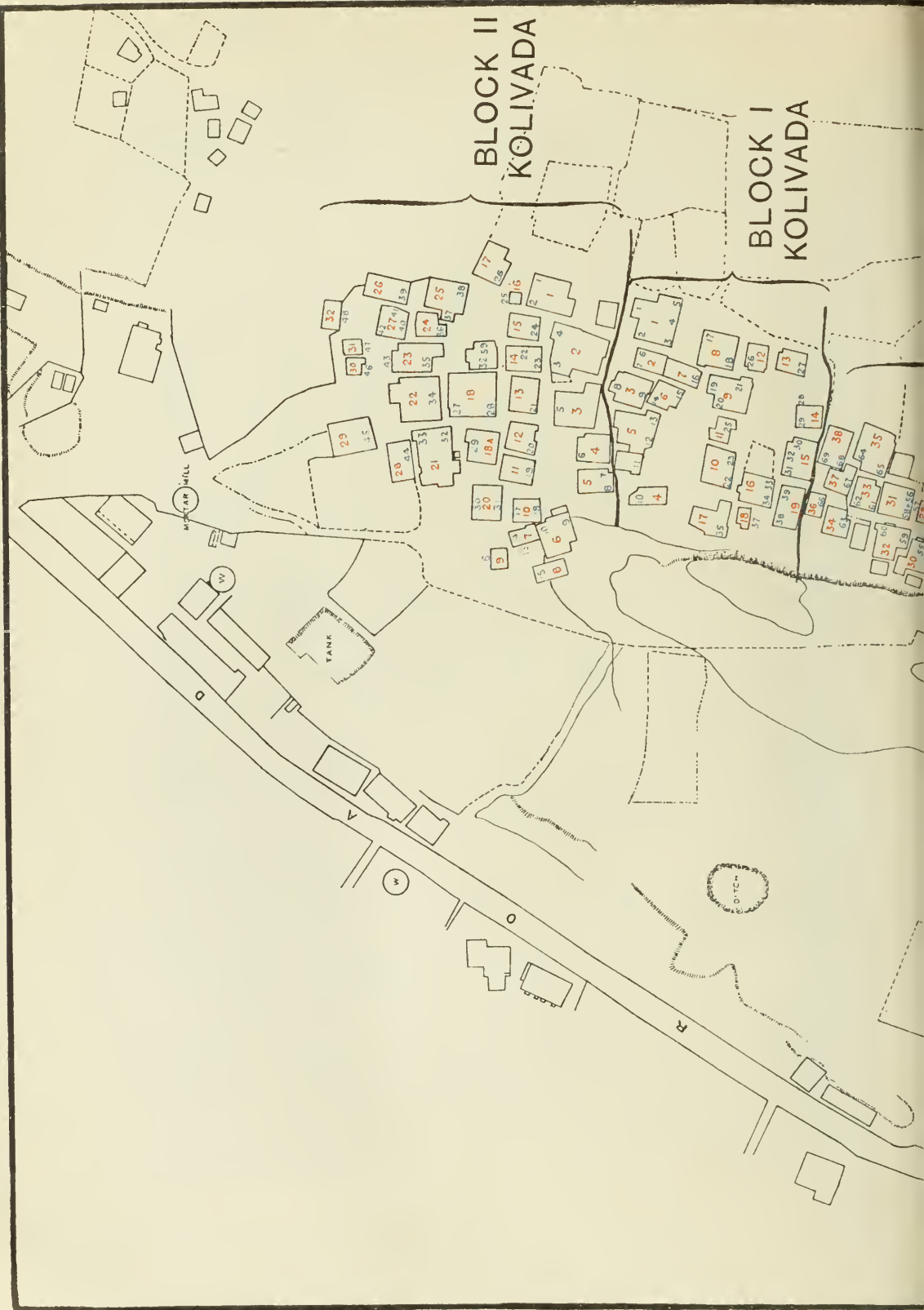
800

SION VILLAGE

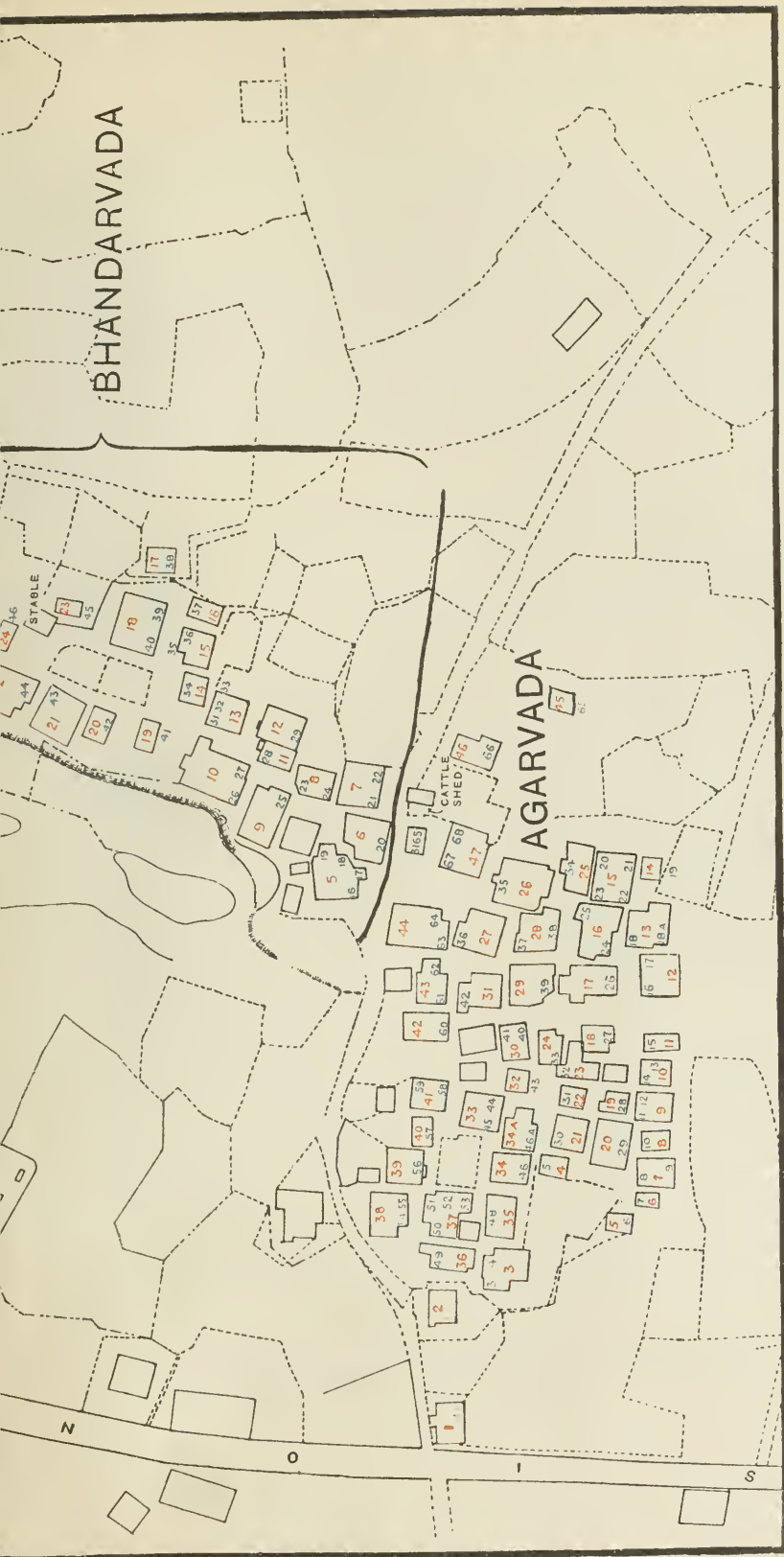
Key to census numbers

BLOCK II  
KOLIVADA

BLOCK I  
KOLIVADA









were greatly increased by the fact that the natives here are much less under the direct influence of their headmen than in the Punjab villages, and that to an equal degree the control of the local authorities over the headmen is in many ways much less personal and effective than in the up-country villages. Still, as the following narrative shows, our efforts in investigating the course taken by plague in these villages cannot be regarded as altogether fruitless.

With this introduction we may now pass on to describe the course of events in each village, dealing first with Sion, in which a most interesting experiment was carried out.

## II. OBSERVATIONS IN SION VILLAGE.

### Section A. *A description of the village in which the observations were carried out.* (Map I.)

#### (a) *Its situation, inhabitants, etc.*

Before entering into details of the observations it is necessary first of all to give a general account of the village in which they took place. This is a small village or hamlet named Sion<sup>1</sup>. It lies in a picturesque situation about eight miles to the north of the European quarter of Bombay City, and about three miles from the northern limit of the island, on the east side of one of the main roads leading from the city to the causeway which connects Bombay with the neighbouring island of Salsette. The village is set somewhat back from the road so that it presents quite an isolated appearance, being surrounded by open country. The inhabitants of Sion at the present day are descendants of the earliest settlers in Bombay.

Nominally, the village is divided from north to south into three nearly equal parts. The most northerly is called Koliwada and is inhabited chiefly by kolis or fishermen; the majority of these are Hindus, but a certain proportion are native Christians. The southernmost part is named Agriwada—literally the village of the Agris. Bounded by Koliwada on the north and by Agriwada on the south, and consequently occupying the middle of the village, there is a part known as Bhandarwada. The aboriginal inhabitants were Bhandaris—a caste, the members of which follow the occupation of toddy drawers. Some of the

<sup>1</sup> The name has been derived from "Simva," i.e. the boundary hamlet—the last inhabited spot before crossing the strait to Salsette. Edwardes, *History of Bombay*, p. 11.

present inhabitants still follow their hereditary calling and may be seen climbing the palm trees in the village and in its vicinity, in order to collect in earthen pots the juice which drips from the incised crown of the trees. Most of them, however, work in the neighbouring fields. As is indicated in the accompanying map (Map I) a ridge of rock of volcanic origin bounds Bhandarwada and a part of Koliwada on the west side. The entire village is built on gently undulating ground—the highest point being at the junction of Koliwada and Bhandarwada.

The population of each part as ascertained by our census was as follows:

Koliwada	...	...	...	375
Agriwada	...	...	...	325
Bhandarwada...	...	...	...	250
Total				950

(b) *Construction of the buildings.*

The construction of the buildings merits attention, since according to our view their peculiar features play a part in the liability of their occupants to plague infection.

A glance at Map I and at Plates XXVIII and XXIX will show that the buildings are isolated from each other and that in no instance are they placed back to back or in rows—in this respect differing entirely from the complex arrangements of the houses in a Punjab village. The great majority have only one story. The outer walls are built of unhewn stones (Plate XXX) cemented together with clay, the foundations extending to a considerable depth below the surface of the ground. The walls are occasionally covered with a layer of limewash. In front of each building there is a verandah (Plates XXX, XXXI), the roof of which is formed of bamboo laths supporting tiles of country make. This verandah is often surrounded on three sides by a low mud wall, and this and its mud floor have a certain finish imparted to them by the periodical application of cowdung.

The level of the floor of the verandah and of the inner rooms is often 4—5 ft. or more above the level of the surrounding ground—a provision which must aid in keeping the houses dry in the monsoon months. The floor of the rooms consists of clay, beaten down till it is firm and smooth, renewed every few years when it becomes uneven, and covered with a layer of wet cowdung, which quickly dries forming a neat and clean surface. The cowdung is renewed at short intervals. The roof consists of country tiles (Plates XXVIII to XXXI), laid upon bamboo





Sion Koliwada, showing the house (1) in which plague began and the site of the health camp (x)







Sion Koliwada: showing houses B, C and F (see text and map IV).





Sion Koliwada: showing tiles, verandah, etc.



Sion Koliwada: House B.







86



Sion Agriwada: House P (see map VII).



laths and slopes upwards on the four sides from the eaves towards a central point.

The internal arrangements in the buildings are simple. The rooms are separated off by lath partitions plastered with mud. There is often a rude loft enclosed within the sloping sides of the roof and used as a general lumber-room. The cooking place, usually surrounded by shining copper and brass pots, occupies a corner of one of the rooms. Another corner is set apart for the reception of the household gods. The people have strong prejudices against natives of another caste and Europeans approaching too near these places, so that in examining the houses it was necessary to respect the feelings of the owners in this regard. Bundles of firewood and cowdung cakes (used as fuel) are stacked in odd recesses, and collections of dried fish and of grain find temporary resting places on the floors.

(c) *Conditions as regards sanitation of the buildings and of the village generally.*

The houses are kept moderately clean, although they usually harbour a good deal of material which is best included in the category of rubbish—the kind of property which is of little apparent use, but which poor people set store by in the hope that some day it may prove useful.

It is difficult, if not impossible, to judge of the adequacy or inadequacy of the ventilation in these houses by the ordinary standards. A mere estimation of the number of cubic feet of air available for each inmate would certainly convey an erroneous idea of the conditions as regards ventilation. It must be remembered that the doors, at least during the day, are kept open and that the windows, consisting of a grid of iron bars with ill-fitting wooden shutters—sometimes with no shutters—are so defective in construction as practically to act as ventilators (Plates XXX, XXXI). The outer walls are often rat riddled and the mud-cement, when it becomes very dry, cracks and gives rise to fissures; in some buildings even large cracks may be seen between the stones of the walls. Lastly, the country-tiled roof forms an admirable ventilator, which is assisted in many houses by large gaps between the top of the outer walls and the eaves of the roof. It seemed to us, in short, that the ventilation of the houses was adequate, and as evidence of this belief it may be noted that on entering any of them we never encountered the disgusting odour due to human emanations, or even the sensation of closeness which is so common a feature of the houses of the very poor in our country.

With regard to the lighting of the houses it must be stated that

many of them are dark but this is surely an advantage in the case of people who for the greater part of the day labour under a fierce and glaring sun.

We fear that the description we have given of mud floors covered with cowdung and of mud-cemented walls conveys to the reader accustomed to live in a substantial house of stone and mortar hermetically sealed against the rigours of a cold and damp climate, an idea of a primitive mode of living with a total disregard for the most ordinary laws of sanitation. And yet these houses give the impression, to one who is familiar with them, that they are excellently adapted to the conditions of life which obtain in a tropical climate. Doubtless in the monsoon months the houses are damp; it would be absurd, nevertheless, to associate the dangers of exposure to damp so familiar to every inhabitant in a country like our own, with the warm and moist conditions that exist in Bombay. Moreover, during the plague season the houses are as dry as a nearly total absence of rain for several months and a tropical sun can render them.

When we come to consider the sanitary conditions which relate to the disposal of refuse, of waste water and of excreta it must be said that in this respect they leave much to be desired. Proper facilities for the disposal of refuse and excreta are entirely absent. As in many other parts of India the people use the neighbouring fields as latrines. Curiously no nuisance is evident from such a method of disposal. The intense heat of the midday sun and the radiation from the hot ground have a powerful desiccating effect on human excreta and quickly render them inodorous.

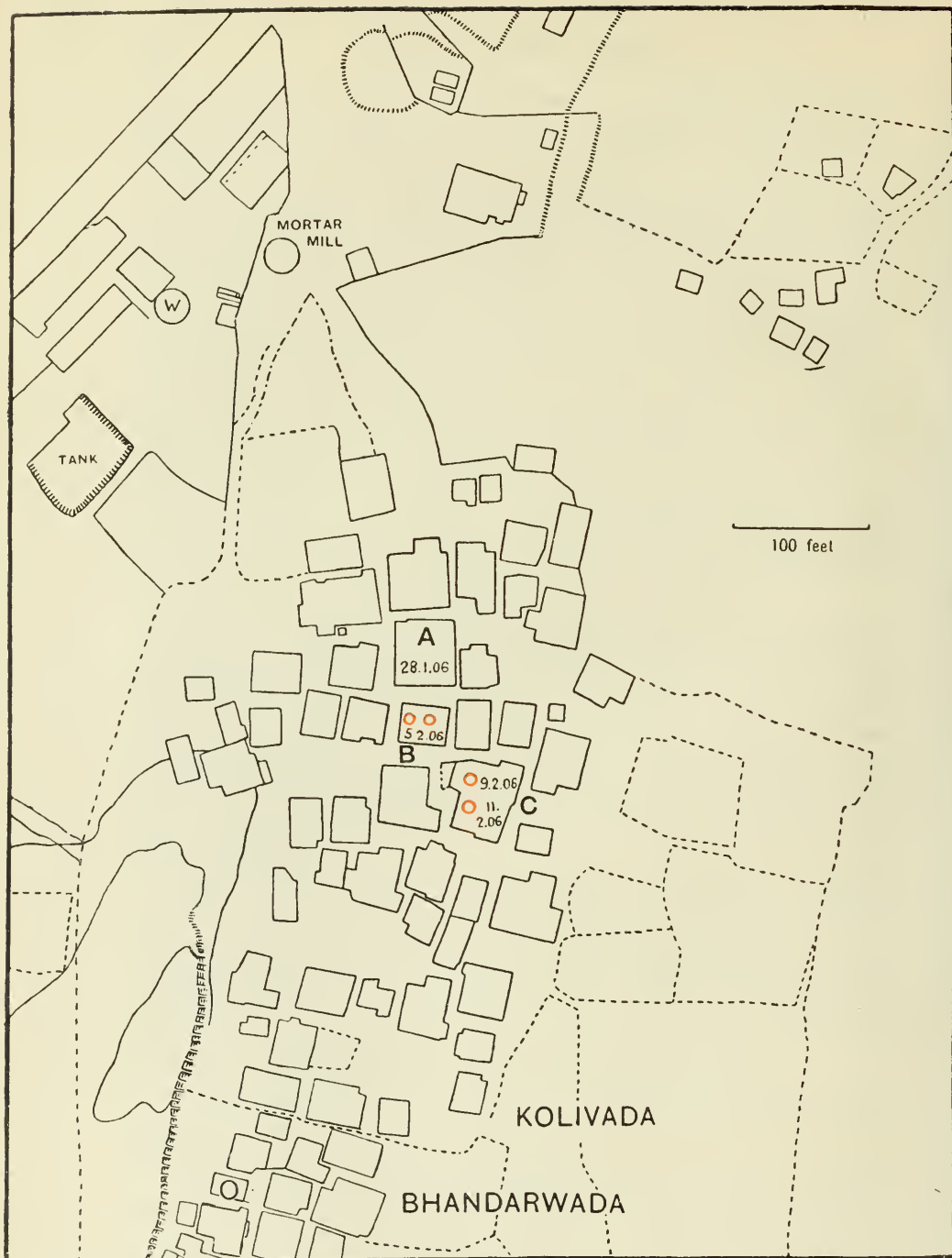
The waste water from the houses finds its way along a channelled stone let through the wall and projecting a foot or so beyond. Occasionally even this simple expedient is dispensed with and the water drips through a hole in the wall from which it drains into the ground or finds its way into a cesspool, formed of a hole about 4 ft. in diameter dug out of the ground and situated a distance of only a few feet from the house. No gullies or drains are to be found in the village.

The water supply is derived from the municipal water pipes and is placed at the disposal of the people by means of stand pipes, situated at one or two convenient spots in the village.

The personal habits of the villagers are on the whole good. Like all Hindus they perform frequent ablutions. Their physique compares favourably with that of natives living in similar circumstances in Bombay City. During the period the village was under surveillance







# SION VILLAGE

Shows events which happened during the second period

○ Date of attack of human case

the incidence of sickness and death from causes other than plague was remarkably light. Though poor the villagers are not poverty stricken, and indeed appear to live a moderately comfortable existence.

Section B. *Observations in Koliwada.*

It will be convenient and will tend to clearness if we divide the events which happened in Koliwada under three headings, namely:

1. The First Period—from the beginning of the investigation in November 1905 to the 28th January 1906.

2. The Second Period—from the 28th January 1906 to the 12th February 1906.

3. The Third Period—from the 12th February 1906 to the end of the experiment, that is, to the 16th April 1906.

A. *Events relating to the First Period, i.e. from November 1905 to the 28th January 1906.*

It has already been mentioned that arrangements were made whereby we might become aware of the first plague rat and of the first human case that occurred in the village. The houses were systematically trapped for rats, traps being set in several houses every day, and the village clerks and their coolies were instructed to keep a look out for dead rats and to remind the villagers of our desire to obtain any rat found dead in their houses. The daily catch of rats caught alive in traps was examined according to exactly the same procedure as the rats brought to the laboratory from the City<sup>1</sup>.

The history of the period we are considering may be dismissed very briefly. So far as we could ascertain the rat population of the village remained free of plague and no human case occurred.

B. *The Second Period—from the 28th January 1906 to the 12th February 1906. (Map II.)*

On a visit to the village on the 29th of January we learned that on the previous day a dead rat had been found in building A<sup>2</sup>. Although

<sup>1</sup> During this period 465 rats were trapped alive from houses in the whole village of Sion. These included 381 *M. rattus*, 81 musk rats and 2 mice. Not a single *M. decumanus* was found.

<sup>2</sup> For ease of description we have departed in the case of certain buildings requiring detailed notice from the nomenclature adopted by us during the investigation. These buildings will hereinafter be referred to by letters of the alphabet given to them in the order of the earliest event occurring in them which pointed to plague infection.

at this time we had no reason to believe that the rat was plague infected yet as a matter of precaution two guinea-pigs were put into the building; they did not contract plague.

On the 7th February some of the villagers informed us in a casual manner that there was a case of plague in one of the houses and that the inhabitants of Koliwada had resolved to evacuate the village, and to go into camp. It was evident that events of importance to us were being withheld and we were naturally disappointed at the distrust shown by the people.

Although it appeared that the main objects of the investigation were frustrated yet it was thought advisable to pursue the inquiry as far as possible. As a result several facts of importance came to light and we are now in a position to state the essential data which pertain to this period. These are briefly:

I. On 28th January the occupants of A according to their own admission found a dead rat in their house and threw it away. On the same day these people and their neighbours in the other house of the building vacated it and went to live in a building a short distance away, namely building C.

II. On 5th February Havloo Akar was attacked with plague in B a house adjoining A; she died on 9th February. Raghunatu Lakshman was attacked with plague on the same day in the same building; he died on 8th February.

III. On 9th February Budhia Sukur was attacked with plague in C; he died on 11th February.

IV. On 11th February Halia Bhiwa, who previous to 28th January lived with his parents in A, was attacked with plague in C; he died on 13th February.

V. The Koliwada villagers began to evacuate the village on 8th February and by the 11th February they had almost all gone into camp.

Although it is impossible to state that the dead rat found in A had died of plague yet at this time we thought it advisable to make inquiries into the matter, as there was a very strong presumption that it was plague infected. Several suspicious circumstances pointed in this direction. We regarded the fact that the families living in A vacated their houses on the day the rat was found as suspicious, because the villagers adopt this precaution when they become aware during the plague season of an unusual mortality amongst the house rats. These considerations, taken in conjunction with the occurrence of several plague cases in the houses adjoining A made us suspect strongly that

the dead rat found in A was plague infected. We cannot put forward the following account of the results of our inquiries into the matter as altogether trustworthy evidence, since it is founded chiefly upon statements made by people who did not give the impression of telling the whole of the truth. It may be cited, however, as an apt instance of the difficulties met with in carrying out epidemiological investigations in Bombay.

On the 16th January 1906 a young woman named Anna Pascal died in house No. 30 Tankbunder Koliwada, a district in Bombay City; the death was registered as "asthma." Her neighbours stated, however, that she suffered from an acute illness with high fever and a bubo in the groin. This woman, Anna Pascal, was a Christian Koli. In the same district there lived another Christian Koli family, named Tukeram, consisting of an old woman named Pasci and her husband and son. When Anna Pascal died the Tukeram family, including the old woman Pasci, attended the funeral. It is necessary in this connection to point out that in Bombay the native funeral ceremonies are for the most part carried out in the room in which the person dies. The friends, especially the women, congregate in the room and assist the relatives in dressing the corpse before it is removed from the house.

On or about the 21st January Pasci paid a visit to her daughter and grandchildren who lived in building A Sion Koliwada. She stayed with her relatives till the morning of the 28th January, the date of the discovery of the dead rat in the house; and then returned to her own house in Tankbunder Koliwada. She became frightened when the dead rat was discovered knowing full well that she had come from an infected locality; she believed that we or the villagers would attribute the infection of the village to her. We could obtain no information from the old woman Pasci as to having brought with her from her own house to her relatives' house A any clothes or other articles likely to contain infection.

It may be admitted that this account offers a plausible explanation of the origin of the rat epizootic which we believed to have originated in A. The information on which the above account is based was derived in the following way. Cross-examination of the owner of A elicited the fact that the old woman Pasci a short time before coming to live with her relatives in A had attended a funeral. We could not extract any definite information as to the address of the woman whose funeral Pasci had attended, except that she lived in Tankbunder Koliwada. Examination of the records of deaths kept by the registrar

in whose district Tankbunder Koliwada is situated showed that the only death likely to have any connection with the case we were investigating was that of the young woman Anna Pascal, although as we have said the cause of death was registered as "asthma." The discrepancy between the death registration and the neighbours' account of the illness does not in the least detract from the likelihood of the illness having been plague, since it is a matter of common knowledge that in Bombay "asthma" is one of the favourite synonyms used by people who wish to conceal a plague death. Subsequent events proved that the clue we were following was the correct one, for when it was put to Pasci that she had attended Anna Pascal's funeral she at once admitted the fact.

*C. The Third Period—from 12th February 1906 to April 1906.*  
(Maps III—VI.)

By the 11th February the Koliwada inhabitants with a few exceptions had removed to a temporary camp in the neighbouring fields, a few hundred yards from the village. This action on the part of the villagers was undoubtedly the outcome of severe lessons learnt during the earlier years of plague in Bombay. For the past four or five years they have been accustomed, acting on their own initiative, to leave the village and to go into camp whenever human deaths associated with dead rats begin to occur. By these people, ignorant as they may be, the significance of a mortality amongst the house rats is thoroughly appreciated. It cannot be doubted that their action in this respect was the best thing that they could do under the circumstances, but it is obvious that by so acting they took away from us all hopes of investigating the relationship of rat and human plague as they may be observed in a non-evacuated village.

We determined, however, to utilise the opportunity experimentally.

Let us now summarise the facts which were at our disposal when the experiment was begun. They were: (1) history of a dead rat found in A; (2) four cases of human plague in the adjoining buildings B and C; (3) an almost completely evacuated village.

There was good reason to suppose that an epizootic had broken out amongst the rats in the buildings in the vicinity of A. Whether this was a limited epizootic or not it was impossible to say, for we had not succeeded in obtaining any plague rats from this quarter during the second period. Still it occurred to us that this point might be tested



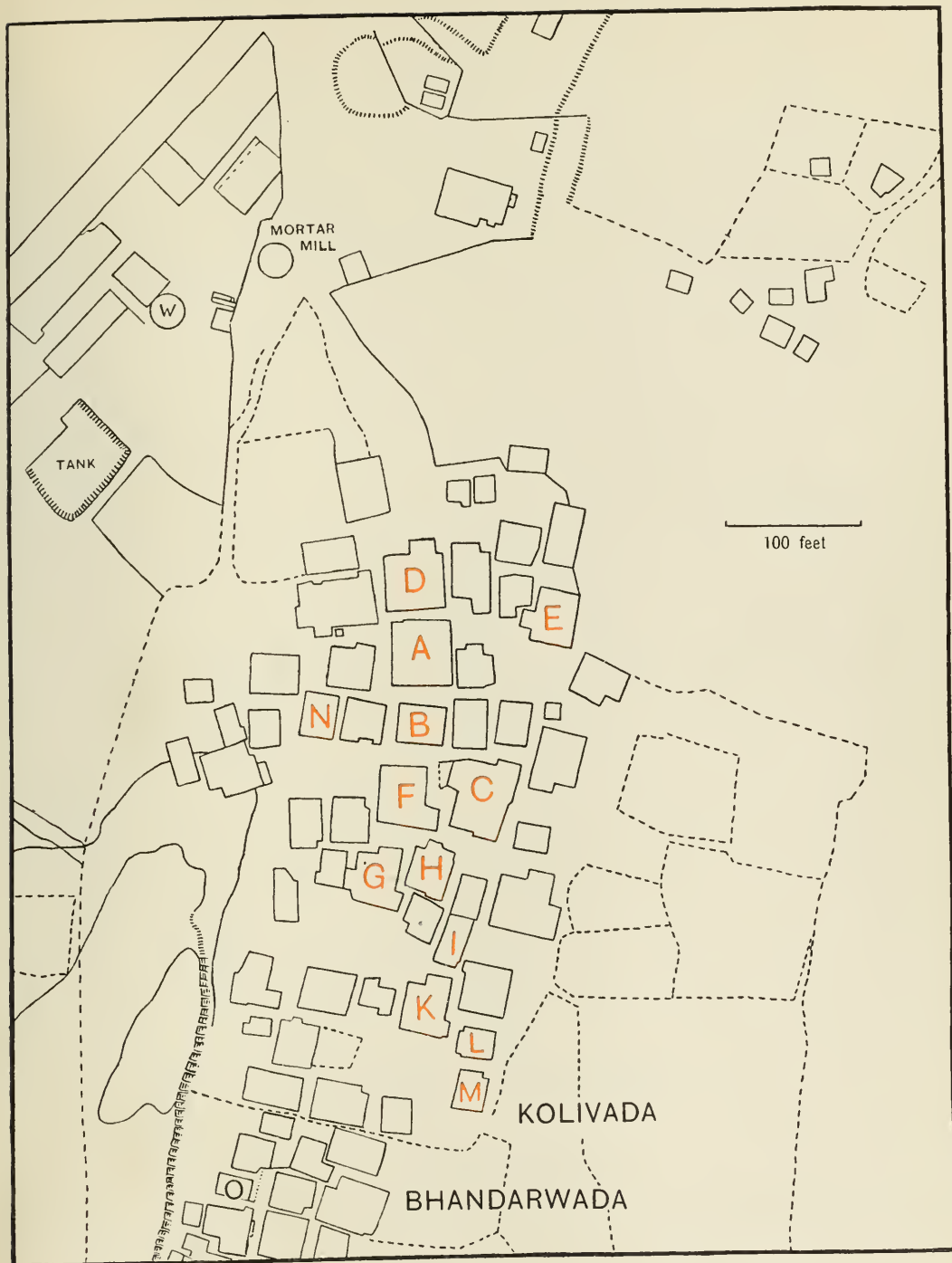


## SION VILLAGE

Shows guinea-pigs which died of plague—only the guinea-pig which died earliest in each house being represented together with the date of death

✕ Guinea-pig died of plague

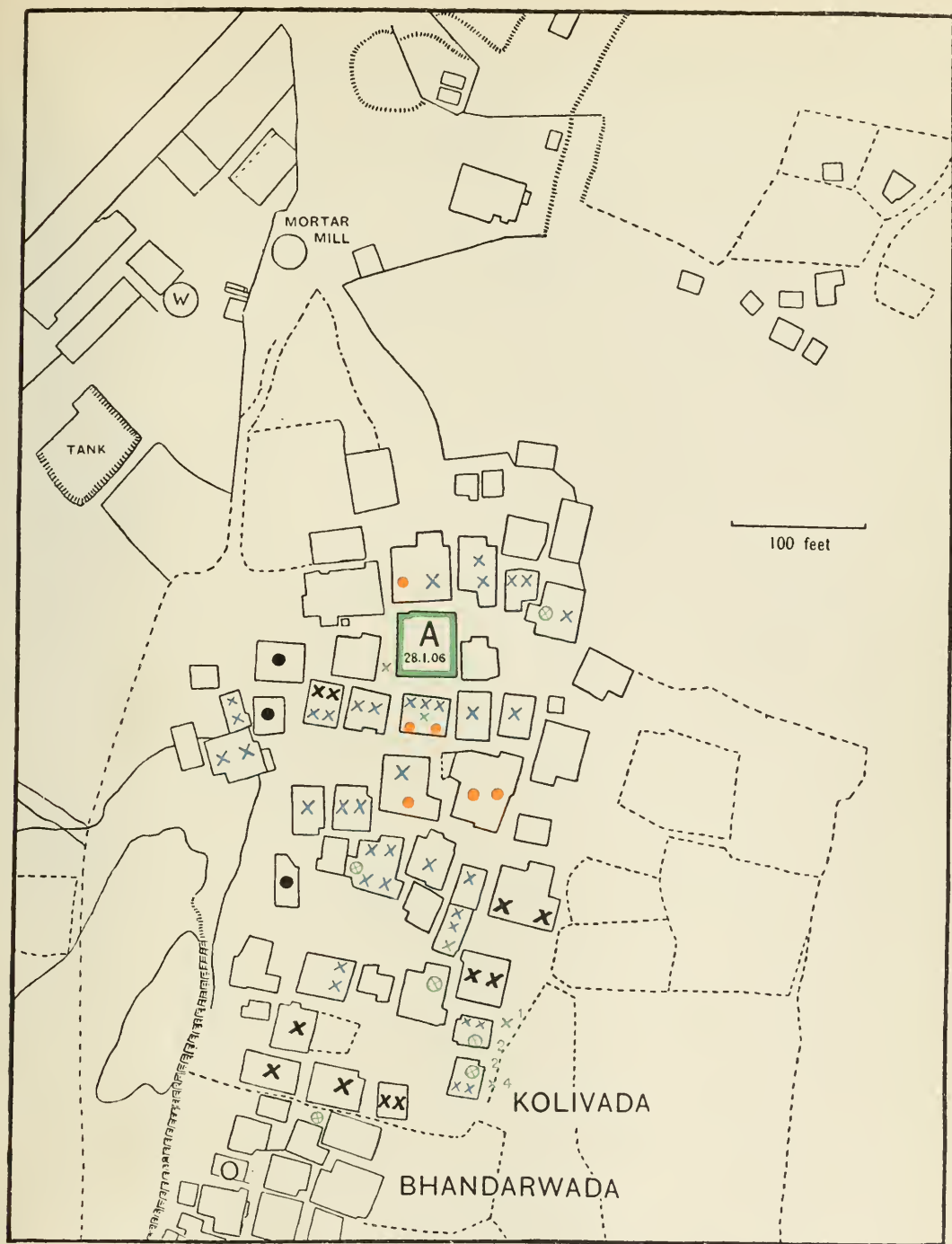




# SION VILLAGE

Key to buildings dealt with under the heading "a detailed description of events requiring special notice which happened in certain houses"





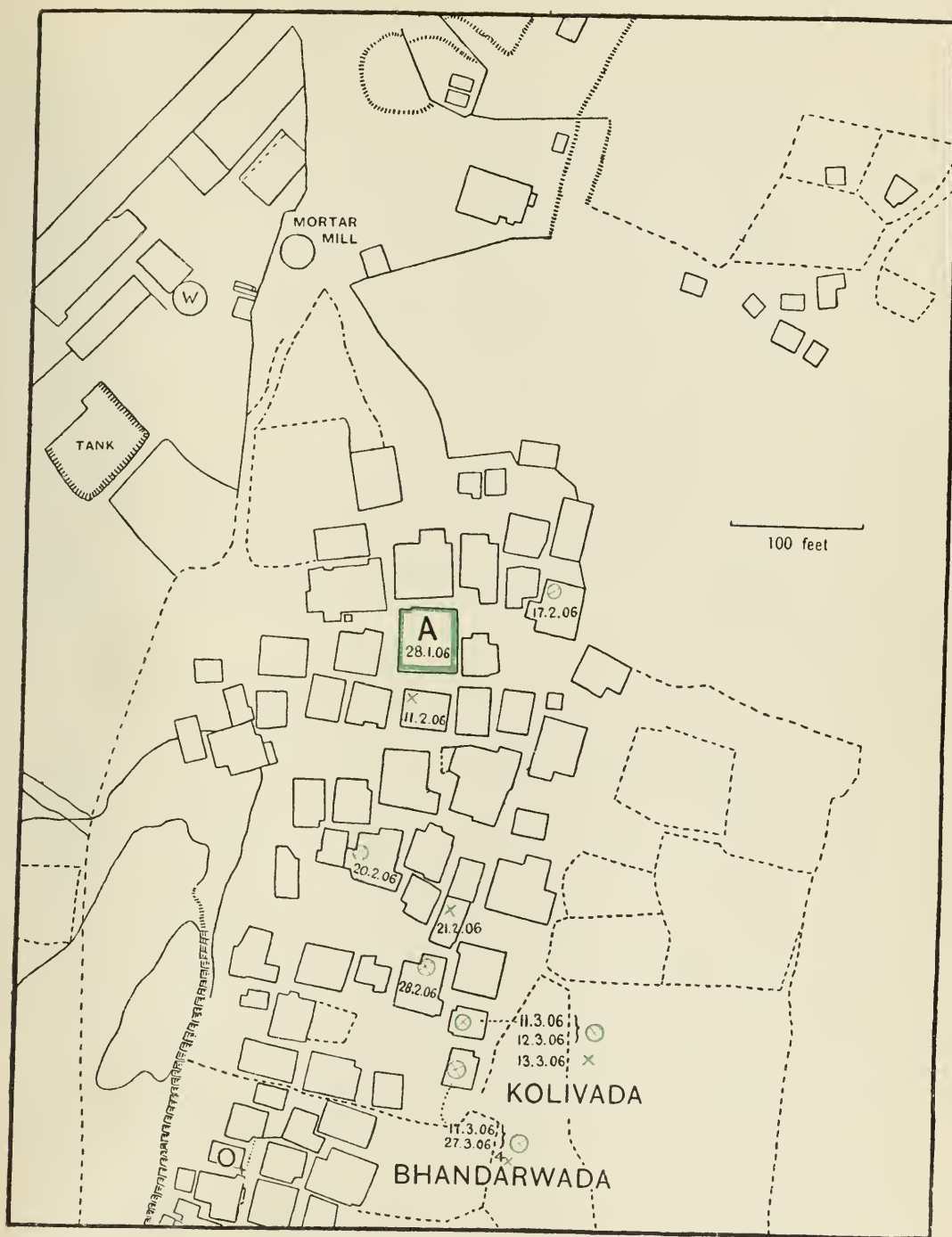
## SION VILLAGE

Shows all the events which happened in the houses

- |                           |                                |                        |
|---------------------------|--------------------------------|------------------------|
| ● Death (human)           | ✕ Guinea-pig dead of plague    | ⊗ Plague rat confirmed |
| ✕ Mummified or putrid rat | ✕ Guinea-pig remaining healthy | ● Non-vacated houses   |







SION VILLAGE

Showing rats found in Kolivada

⊗ Undoubted plague

✕ Mummified or putrid



by substituting in the houses throughout the village a guinea-pig population. The advantages of such a procedure were obvious: 1. It would be an easy matter to examine the guinea-pigs for the presence of rat fleas. 2. It suggested itself that it might be possible to establish a relationship between plague rats found by search in the houses, and any plague infection which might become evident amongst the guinea-pigs. 3. The outstanding advantage of such a scheme appeared to be the circumstance that the guinea-pigs were entirely under our control, that they could be kept confined and isolated from each other in the buildings, and that if an epidemic should break out amongst them the objection certainly could not be urged that a guinea-pig in one building had contracted its infection directly from a guinea-pig living in another building.

Briefly it may be said that this scheme was carried out and that it proved successful beyond our expectations.

As the details of the experiment are of necessity somewhat confusing since frequently several events happened at nearly the same time in houses in different parts of the village, we propose in the following account to arrange them under several headings.

1. *An account of the methods adopted in the experiment.*

On the 12th February two guinea-pigs were allowed to run free in each of seven vacated buildings in the immediate neighbourhood of A and as far as possible surrounding it. Within the next few days the range of buildings into which guinea-pigs were put was extended, so that by the 15th February most of the houses in the village sheltered two guinea-pigs. Difficulties were experienced at the outset; for example, we found on visiting, 24 hours later, the first houses into which guinea-pigs had been placed, that in some of them either one or both the animals had been killed by cats. On this account it was found necessary to place the animals in wire cages on the floor of the houses. Again, in some instances we could not gain access to the houses, either because their occupants could not be traced or because they were unwilling that we should enter them. Further, a few houses in the village remained occupied throughout the period of the experiment, so that it seemed unnecessary to add guinea-pigs as their human occupants served as a control.

The animals were examined every day—in the morning and evening—and when a favourable opportunity arose a search was made in the

TABLE I.

*Giving the details of guinea-pigs (running about free or in wire cages) which died of plague in the houses in Koliwada. They are arranged with reference to the earliest date of death of a guinea-pig in each house.*

Serial No.	House No.	Date when guinea-pig was put in	Date of death	No. of fleas on dead guinea-pig, or before removal to laboratory	Bubo	Remarks
1	II. 13. 21 (B)	10. 2. 06	13. 2. 06	42	Submax.	—
		15. 2. 06	20. 2. 06	0	"	
		15. 2. 06	21. 2. 06	12	"	
2	II. 12. 20	12. 2. 06	17. 2. 06	3	"	—
		12. 2. 06	19. 2. 06	64	"	
3	II. 23. 35	12. 2. 06	17. 2. 06	0	"	—
		12. 2. 06	18. 2. 06	16	"	
4	II. 24. 36	13. 2. 06	17. 2. 06	15	"	—
		13. 2. 06	21. 2. 06	18 rat fleas 1 cat flea	"	
5	II. 25. 37 (E)	14. 2. 06	17. 2. 06	8	"	—
6	I. 2. 7	13. 2. 06	18. 2. 06	5	Rt. submax. Lt. inguinal	—
7	II. 3. 5 (F)	12. 2. 06	18. 2. 06	0	Submax.	Guinea-pig found dead partly eaten by rats.
8	II. 6. 10	15. 2. 06	19. 2. 06	0	"	—
		15. 2. 06	1. 3. 06	1 rat flea 1 cat flea	"	
9	II. 14. 23	12. 2. 06	19. 2. 06	89	"	—
10	II. 22. 34 (D)	12. 2. 06	19. 2. 06	18	"	—
11	I. 5. 13 (G 2)	14. 2. 06	20. 2. 06	0	"	—
		14. 2. 06	22. 2. 06	2	"	
12	II. 4. 6	15. 2. 06	20. 2. 06	0	"	—
		15. 2. 06	20. 2. 06	14	"	
13	I. 5. 12 (G 1)	14. 2. 06	21. 2. 06	6 cat fleas 3 rat fleas	"	—
		14. 2. 06	22. 2. 06	4	" Rt. inguinal	
14	I. 10. 23	15. 2. 06	21. 2. 06	1	Submax.	—
		15. 2. 06	21. 2. 06	3	"	
15	II. 5. 7	14. 2. 06	21. 2. 06	23 rat fleas 1 cat flea	"	—
16	I. 3. 9 (H)	13. 2. 06	22. 2. 06	12 rat fleas 2 cat fleas	"	—
17	II. 15. 24	12. 2. 06	22. 2. 06	0	"	—
18	II. 7. 14	15. 2. 06	23. 2. 06	4	"	Chronic plague (relatively).
		15. 2. 06	28. 2. 06	3	"	
19	I. 7. 16 (I)	15. 2. 06	24. 2. 06	8	"	—
		15. 2. 06	3. 3. 06	0	"	
20	I. 13. 27 (M)	15. 2. 06	10. 3. 06	0	No definite bubo	Culture from heart-blood. Chloroformed to death.
		15. 2. 06	4. 4. 06	4	Lt. inguinal	
21	I. 12. 26 (L)	15. 2. 06	18. 3. 06	8	Submax.	Submax. phlyctenule.
		15. 2. 06	22. 3. 06	26	"	
22	II. 11. 19 (N)	14. 2. 06	19. 3. 06	0	"	—
		14. 2. 06	23. 3. 06	1	"	



houses for dead rats. The odour of a putrefying rat is quite distinctive and this on more than one occasion led to a search, which resulted in the discovery of one or two dead plague rats. The guinea-pigs were frequently examined for fleas, and in every case those which had a bubo or appeared to be sick were chloroformed for this purpose. The fleas were removed, a note was made of the number and species, and they were then, as a rule, put back into the house. Every sick guinea-pig, after being cleared of fleas, was removed to the laboratory for segregation. The object of this precaution was to prevent the fleas becoming infected from the sick animal when a septicaemia supervened, because otherwise its companion, if still healthy, might contract plague from such infected fleas.

2. *A general description of the epidemic which broke out amongst the guinea-pigs placed in the houses.*

The details of the guinea-pig epidemic have been arranged in Table I and they have also been represented graphically on Map III. The course of events will be best appreciated by a study of the map so that we are exempted from the necessity of describing in detail the progress of the epidemic. The reader will readily perceive that our conjecture proved correct, that widespread infection throughout the village had occurred, probably originating in A.

It will, we think, be granted also that a review of the table leads to the conclusion that the infection spread peripherally from the focus A, especially towards the southern boundary of Koliwada, that is, the part contiguous to Bhandarwada. For full details of all the guinea-pigs which died of plague the reader is referred to Table I.

3. *A detailed description of events requiring special notice which occurred in certain houses.*

In order that the account may be consecutive and thus more easily understood, the houses coming under this heading will be dealt with in the order of the earliest event occurring in them which pointed to the presence of plague infection.

*Building II. 18. 27, 28 (A)<sup>1</sup>.*

This building was occupied by two families, both of them Christian Kolis. Since the events which happened in it are of special importance

<sup>1</sup> *i.e.* Block II, building 18, houses 27 and 28; this building being also distinguished as A.

the circumstances connected with it may be briefly recalled. On or about the 21st January the old woman Pasci came from her house in Tankbunder Koliwada in Bombay City and lived here for a week with her daughter and grandchildren. It is probable that she brought with her an infecting agent, at the nature of which we can only guess, acquired perhaps during her attendance at the funeral ceremonies of Anna Pascal, who in all likelihood died of plague. It is interesting to note that, assuming this explanation to be a right one, Pasci herself did not suffer from plague nor in the first instance did any of the occupants of this building.

There can be no doubt that the occupants of A found a dead rat in the house on 28th January, and it is evident that they suspected this rat of being plague infected, for they and their neighbours in the same building vacated their houses on the same day and went to live in C.

TABLE II.

*Giving details of guinea-pigs placed in houses in Koliwada which remained healthy till the end of the experiment. They are arranged in order of the House Number.*

Serial No.	House No.	Date when put in	Remarks
1	I. 1. 3 & 5	I. 1. 3, 2 guinea-pigs 14. 2. 06 I. 1. 5, 2 guinea-pigs 15. 2. 06	—
2	I. 3. 9 (H)	1 guinea-pig 13. 2. 06	Removed on 20. 2. 06 to laboratory for isolation together with a sick guinea-pig which died of plague.
3	I. 8. 17 & 18	2 guinea-pigs in cage 18. 3. 06	—
4	I. 14. 29	2 guinea-pigs 15. 2. 06	—
5	I. 15. 31	1 guinea-pig in cage 23. 2. 06	—
6	I. 16. 33	1 guinea-pig in cage 23. 2. 06	—
7	I. 19. 39	1 guinea-pig in cage 23. 2. 06	—
8	II. 5. 7	1 guinea-pig 14. 2. 06	Removed on 20. 2. 06 to laboratory for isolation together with a sick guinea-pig which died of plague.
9	II. 11. 19 (N)	2 guinea-pigs 12. 2. 06	Note :—2 other guinea-pigs in this house put in on 14. 2. 06 died of plague on 19. 3. 06 and 23. 3. 06.

*Diary of events prior to the experiment.*

28. 1. 06. A dead rat was said to have been found and thrown out of (A) on this date. The inhabitants of (A) left their houses and went to live in (C).  
 5. 2. 06. Hayloo Akar was attacked with plague in (B). He died on 9. 2. 06.  
 Raghunatu Lakshman was attacked with plague in (B). He died on 8. 2. 06.  
 9. 2. 06. Budhia Sukur was attacked with plague in (C). He died on 11. 2. 06.  
 11. 2. 06. Halia Bhiwa (of II. 18. 27) was attacked with plague in (C). He died on 13. 2. 06.

TABLE III.

*Showing Koliwada houses arranged in order of date of the earliest event occurring in them, which pointed to plague infection being present in the house.*

Serial No.	House No.	Date of earliest event	Date on which guinea-pig was put into house	Interval between date of putting in guinea-pig and its death	Remarks
1	II. 18. 27 (A)	28. 1. 06	—	—	History of dead rat found.
2	II. 13. 21 (B)	5. 2. 06	—	—	Havloo Akar and Raghunatu Lakshman attacked with plague.
3	II. 2. 3 & 4 (C)	9. 2. 06	—	—	Budhia Sukur attacked with plague.
4	II. 22. 34 (D)	14. 2. 06	—	—	Maribai Amber attacked with plague.
5	II. 12. 20	17. 2. 06	12. 2. 06	5 days	Guinea-pig dead of plague.
	II. 23. 35		12. 2. 06	5 "	" " "
	II. 24. 36		13. 2. 06	4 "	" " "
	II. 25. 37 (E)		14. 2. 06	3 "	In II. 25. 37 plague rat found.
6	I. 2. 7	18. 2. 06	13. 2. 06	5 "	Guinea-pig dead of plague.
	II. 3. 5 (F)		12. 2. 06	6 "	" " "
7	II. 6. 10	19. 2. 06	15. 2. 06	4 "	" " "
	II. 14. 23		12. 2. 06	7 "	" " "
8	I. 5. 12 (G 1)	20. 2. 06	—	—	Plague rat found.
	I. 5. 13 (G 2)	—	14. 2. 06	6 days	Guinea-pig dead of plague.
	II. 4. 6		15. 2. 06	5 "	" " "
9	I. 10. 23	21. 2. 06	15. 2. 06	6 "	" " "
	II. 5. 7		14. 2. 06	7 "	" " "
10	I. 3. 9 (H)	22. 2. 06	13. 2. 06	9 "	" " "
	II. 15. 24		12. 2. 06	10 "	" " "
11	II. 7. 14	23. 2. 06	15. 2. 06	8 "	" " "
12	I. 7. 16 (I)	24. 2. 06	15. 2. 06	9 "	" " "
13	I. 9. 19 (K)	28. 2. 06	—	—	Plague rat found.
14	I. 13. 27 (L)	10. 3. 06	15. 2. 06	23 days	Guinea-pig dead of plague.
15	I. 12. 26 (M)	11. 3. 06	—	—	Plague rat found.
16	II. 11. 19 (N)	19. 3. 06	14. 2. 06	23 days	Guinea-pig dead of plague.

We became aware on the following day that a dead rat had been found, and although at this time we had no reasons for suspicion, yet as a matter of precaution two guinea-pigs were put into the building and on the following day were examined for fleas. One of them yielded 14 rat fleas and the other 10. The guinea-pigs were brought back to the laboratory for isolation but remained healthy.

On the 12th February two guinea-pigs were put into A but they could not afterwards be found. We had difficulty in obtaining the keys of these houses, and for this reason further experiments could not be carried out.

On the 27th March, that is towards the end of the experiment, the skeleton of a *Mus rattus* was found in some rubbish on the verandah of the building. It ought to be added that this building, compared with other buildings in the village, is built in a substantial manner.

*House II. 13. 21 (B) (Plates XXIX, XXX).*

The first cases of plague in the village occurred in this house. As we have already explained they came to our notice unexpectedly.

On the 5th February a girl aged 16 years, named Havloo Akar, a Sonkoli by caste, was attacked with plague. During her illness she was removed to camp and died there on 9th February. No examination was made during life on account of the concealment of the case, but the body was examined after death and a bubo was found in the left groin. The second case in this house was that of Raghunatu Lakshman, a boy seven years old, and a Maratha by caste. He also was attacked on the 5th February and died three days later. The body was examined after death and buboes were found on both sides of the neck.

The occupants of the house denied having found dead rats but a mummified rat was found by us on 11th February. This building immediately adjoins A on its south side. It has a very broken-down appearance, the outer walls being riddled with rat holes and one corner bulging outwards and showing large cracks, as if due to subsidence of the foundations.

The following experiments were carried out in the house :

(1) On the morning of the 9th February two guinea-pigs were allowed to run free in it and at 5 p.m. of the same day they were examined for the presence of fleas. On one 18 fleas were found and on the other five. These 23 fleas were removed to the laboratory and were transferred to a white rat in a flea-proof cage; this rat did not develop plague. The guinea-pigs were returned to the house and were examined next morning; one of them then yielded five fleas and the other 12. The guinea-pigs were then removed to the laboratory and segregated, but they remained healthy.

(2) On the 10th February four guinea-pigs were placed in the house. After 24 hours the first yielded 42 fleas: these were put on a guinea-pig in a flea-proof cage in the laboratory. This guinea-pig remained healthy but the guinea-pig which yielded the fleas for the cage experiment died of plague on the 13th February, after removal to the laboratory for isolation. The second and third guinea-pigs which

were put into the house yielded between them 37 fleas. These fleas were brought to the laboratory and were put along with a guinea-pig in a flea-proof cage; this guinea-pig, however, remained healthy as did also the animals which furnished the fleas for the experiment. The fourth guinea-pig which was put into the house could not be found.

(3) On the 13th February two guinea-pigs were introduced into the house. Next day 43 fleas were obtained from them. The fleas were removed to the laboratory in the evening and were put next morning on a guinea-pig in a flea-proof cage; this animal however was not attacked with plague. The guinea-pigs which furnished the fleas had disappeared on the 15th February.

(4) On the 15th February, two guinea-pigs were allowed to run free in the house. On the 18th they were put in a wire cage. Next day both were found to be sick and buboes could be felt in the neck. On the following day they were removed to the laboratory, where one of them, on which no fleas had been found before removal, died of plague on the 20th. The other, which furnished 12 fleas before removal, died of plague on the 21st.

*Building II. 2, 3, 4 (C) (Plate XXIX).*

It will be remembered that on the 28th January, the day on which they found the dead rat, the families in A took up their abode in this building. On the 9th February a man named Budhia Sukur, aged 40 years, and *belonging to the family originally living in the house C*, was attacked with plague. He was examined before death by the registrar of the district, Dr Bhatavadekar, who reported that the patient had a temperature of  $102.5^{\circ}$ , a dry and fevered tongue, injected conjunctivae, and that he was undoubtedly suffering from plague; there was a right femoral bubo. This man died on 11th February.

On 11th February a boy 16 years old, named Halia Bhiwa, was attacked with plague. He was a grandchild of Pasci and formerly lived with his mother in A. Dr Bhatavadekar examined him before death and found a right femoral bubo, with symptoms of high temperature, fevered tongue and injected eyes. In Dr Bhatavadekar's opinion the boy was suffering from plague. The boy died on the 13th February.

With regard to the source of infection of the cases in this building and in B, it is important to point out that the people in C were Christian Kolis, while those living in B were Hindu Kolis. It is certain that on account of this difference in caste these people had no connection with



each other. One is driven to conclude then that the common source of infection was the epizootic which probably originated in A.

It is possible to give several explanations of the method of infection in the case of the man and the boy living in C. It is conceivable, for example, that the rats in the house became infected in the course of the epizootic or from infected rat fleas introduced by the occupants of A in their belongings. Another possible explanation is that such infected fleas introduced into the building may have directly infected the persons who developed plague. Judging from the dates of attack of the cases in B and of those in this building the most probable explanation is the one given first, namely, that the infection was derived from the rats in the building, which had become plague infected in the course of the epizootic. In this connection it may be noted that Budhia Sukur was attacked several days before Pasci's grandchild.

The guinea-pig experiments in this building proved to be unsatisfactory. On the 12th February two guinea-pigs were placed in the building. Next day they yielded 10 rat fleas and 28 cat fleas. The rat fleas were removed to the laboratory and were put on a white rat in a flea-proof cage; the rat died in 10 days but the cause of death was not plague. On the 14th the guinea-pigs could not be found. On the same day they were replaced by two more guinea-pigs but next day these also had disappeared. No further experiments were carried out.

*Building II. 22. 34 (D).*

This building immediately adjoins A on its north side. The events connected with it which require notice are:

- (1) a positive guinea-pig experiment, and
- (2) a case of human plague.

*Experiment:* on the 12th February two guinea-pigs were put into the house. On the 17th one of them was noted to be sick: 18 fleas were secured on this guinea-pig and it was then brought to the laboratory for isolation, where it died of plague on the 19th. The other guinea-pig could not be found.

On the 14th February Maribai Amber, a woman 35 years old and a Christian Koli by caste, was attacked with plague; she died in a hut in the camp after an acute illness. The body was examined after death and a bubo was found in the right groin. This woman and the rest of the people living in the house vacated it on the 2nd February. There

can be little doubt, however, that she contracted the infection in this house, for the women of the camp were in the habit of visiting their houses in the village during the day time for purposes connected with their household duties, thus rendering themselves liable to the risk of infection.

*House II. 25. 37 (E).*

This house is owned by the "patel" (headman) of the village. It is situated in a row of buildings to the north of A. The incidents relating to this house which deserve notice may be summarised thus:

- (1) the discovery of a plague-infected rat in the house,
- (2) a positive guinea-pig experiment,
- (3) a case of plague whose infection was possibly contracted in the house.

(1) A strong smell of "dead rat" was noticed in the house on 17th February and on search a dead *Mus rattus* was found. Since on post-mortem examination it proved to be too putrid to permit of a diagnosis being made, the heart-blood and spleen were inoculated cutaneously on a guinea-pig, which subsequently died of plague.

(2) *Guinea-pig experiment*: on the 14th February two guinea-pigs were put into the house. On the 17th one was observed to be very sick; eight fleas were secured from it. It died shortly after removal to the laboratory and on dissection presented typical appearances of plague. The other guinea-pig appeared to be well. A third guinea-pig was placed in the house on the 17th, but of the two which now remained one was found partly eaten next day while no trace of the other could be discovered.

(3) On the 11th March a boy eight years old was attacked with plague. He lived with his uncle, the owner of this house, in a hut in the camp. A right femoral bubo developed and the boy died after a very acute illness. It is impossible in this case to state definitely the source of infection. We can only point out that the boy was in the habit of visiting this house with his aunt during the day time and it is possible that he derived the infection during one of these casual visits. We know at least that infection was present in the house three weeks previous to the date of his attack.

*House II. 3. 5 (F) (Plate XXIX).*

This house is situated directly opposite B, in which the first two plague cases occurred, and adjoins C which, as has already been described, also furnished two plague cases.

The following events connected with it require notice :

- (1) a positive guinea-pig experiment,
- (2) a case of plague whose infection was probably contracted in this house.

(1) *Guinea-pig experiment*: on the 12th February two guinea-pigs were placed in the house. On the 18th one was found dead and partly eaten; post-mortem examination showed typical appearances of plague. The other guinea-pig could not be found.

(2) On the 24th February Godibai Moti, a woman 40 years old and a Sonkoli (Hindu) by caste, was attacked with plague while living in the camp. We examined the patient and found that she had a high temperature, injected eyes, the typical speech of plague, and a right femoral bubo. She died on the 1st of March. Although she had been in the camp for 15 days there seems little room for doubt that she contracted the infection during a visit to this house for domestic purposes.

*Building I. 5. 12, 13 (G 1 and G 2).*

This building adjoins the south wall of the house just described and is separated from it by a narrow lane. The events which happened in it may be summarised thus :

- (1) discovery of plague-infected *Mus rattus*,
- (2) death from plague of two guinea-pigs in the house G 1,
- (3) death from plague of two guinea-pigs placed in the house G 2.

(1) On the 20th February a search was made in the house for rats and as a result a dead *Mus rattus* was found on the floor of one of the rooms of G 1. It yielded 12 cat fleas and 10 rat fleas. Examination proved that it was plague infected.

(2) On the 14th February two guinea-pigs were allowed to run free in the house G 1, and on the 18th they were put into a wire cage placed on the floor. On the 20th both were discovered to be sick. One of them had a submaxillary bubo and after removal to the laboratory for isolation died of plague on the 22nd. The second animal also had a bubo, and six cat fleas and three rat fleas were obtained from it. It was removed to the laboratory where it died of plague on the 21st.

On the 14th February two guinea-pigs were allowed to run free in the house G 2, and on the 18th they were transferred to a cage placed on the floor. On the 20th one was found dead of plague but no fleas were got on it. The other yielded two fleas; it was removed to the laboratory and died of plague on the 22nd February.

House I. 3. 9 (H).

This house occupies a position contiguous to G 2 and F. It furnished a successful guinea-pig experiment.

On the 13th February two guinea-pigs were allowed to run free in the house, and on the 18th they were confined in a wire cage. On the 20th one of them was sick and had a submaxillary bubo; two cat fleas and 12 rat fleas were secured from it. It was kept segregated in the laboratory and died of plague on the 22nd February. The other animal was thought to be somewhat sick and was therefore removed to the laboratory but it remained healthy. Six rat fleas and one cat flea were caught on it before removal.

House I. 7. 16 (I).

This house is not far distant from H but is somewhat nearer the southern limit of Koliwada. Attention may be directed to two events of interest connected with it, namely,

- (1) the discovery of a mummified rat in the house, and
- (2) a successful guinea-pig experiment.

(1) On the 21st February the characteristic rat odour was noticed; the house was therefore searched for rats but only the mummified remains of a *Mus rattus* were discovered.

(2) On the 15th February two guinea-pigs were allowed to run free in the house. They were kept confined in a wire cage after the 18th. On the 22nd one flea was caught on one of the guinea-pigs and a bubo was felt on the other. The latter animal yielded 8 fleas. It was removed to the laboratory and died of plague on the 24th February. On the 3rd March the remaining guinea-pig was found dead of plague.

House I. 9. 19 (K).

This house is a building which is situated immediately to the south of the one just described. The occupants vacated it in order to go into camp on the 12th February, but we were unable to use the house for an experiment for several weeks because the key was not available. The following events of interest occurred in connection with this house:

- (1) the discovery of a plague-infected *Mus rattus*,
- (2) a laboratory experiment with fleas obtained from this rat,
- (3) an experiment with guinea-pigs in specially constructed cages placed within the house.



(1) On the evening of the 28th February an adult *Mus rattus* was captured and killed, as it was coming out of an opening in the wall intended for the exit of waste water. Post-mortem examination proved the rat to have typical septicaemic plague, cultures of *B. pestis* being obtained from the heart-blood, spleen and liver. Immediately after its capture 12 rat fleas were secured from it.

(2) These fleas were at once brought to the laboratory and, after being fed on a shaved area of the abdomen of a guinea-pig, were put with it into a flea-proof cage. The guinea-pig, however, did not develop plague.

(3) On the 1st of March the whole of the building was searched in a thorough manner, but no dead rats were found. On this date also two guinea-pigs were put into the house, one in a cage with wire gauze curtains affording protection of the inmate against fleas and the other in a similar cage but without this protection. The cages were kept in the house for 11 days and the guinea-pigs were then removed and isolated, but they both remained healthy. Although frequent examinations of the animals were made during their sojourn in the house on no occasion were any fleas found upon them.

#### *House I. 12. 26 (L).*

This is a small house situated quite near the last and only a short distance from the southern boundary of the village (Koliwada). The events which occurred in connection with it are of particular interest and importance in their relation to the entire experiment. We propose to deal with them under three headings:

- I. An account of the dead rats found in the house.
- II. The routine guinea-pig experiment carried out in the house.
- III. An account of special experiments which were carried out.

##### *I. An account of the dead rats found in the house.*

On the 11th March during a thorough search in the house for dead rats an adult *Mus rattus* showing signs of putrefaction was found. No fleas were captured on it. It had a right axillary bubo, containing swarms of plague bacilli including involution forms, and in addition many *B. pestis* were found in the spleen and heart-blood. Next day a dead *Mus rattus* was found on the floor near the door: 25 rat fleas were caught on it. This rat had a right submaxillary bubo and a culture of *B. pestis* was obtained from the heart-blood. On the following day a dead *Mus rattus* was found under a pile of firewood. It was very



maggoty so that it was impossible to make anything out of the post-mortem appearances, but a few plague-like bacilli were seen in the spleen smear together with many putrefactive bacilli, and many plague-like bacilli were seen in a preparation of the heart-blood.

## II. *The routine guinea-pig experiment.*

On the 15th February two guinea-pigs were put into the house. On the 16th March, *i.e.* a month later, one of the guinea-pigs showed a submaxillary bubo. At least five fleas were seen on this animal and three or four on the other. Two days later one of the guinea-pigs was found dead and eight fleas were secured from it. Examination revealed deep cervical buboes and other typical appearances of plague. The second guinea-pig yielded 26 fleas; these were put back into the house. This animal was brought to the laboratory for segregation and died on the 22nd March, the post-mortem showing neck buboes and other signs of plague. It is interesting to note that this guinea-pig during life had a small phlyctenule in the submaxillary region. It is noteworthy that in this house a plague rat was found five days before the guinea-pigs showed signs of illness.

## III. *Special experiments carried out in connection with this house.*

I. On the 12th March the following animals were placed in the house:

- (1) A monkey in a cage protected from fleas with fine wire gauze, and a monkey in a similar cage without gauze.
- (2) A white rat in a tanglefoot cage, and a white rat in a similar cage without tanglefoot.

All these animals were kept in the house for two days and were then brought to the laboratory for isolation.

Three fleas were got on the unprotected monkey. No fleas were obtained before removal on the rat in the tanglefoot cage but seven fleas were got on the tanglefoot. It was found on dissection of these fleas that five of them contained bacilli in their stomachs resembling *B. pestis*. On the control rat two fleas were caught, one of which was found to contain similar bacilli. Both rats, however, remained healthy. Both monkeys, also, remained perfectly healthy.

II. 25 fleas captured on the rat which was found in the house on the 12th March and which was proved to be plague infected, were brought to the laboratory and put on a guinea-pig in a flea-proof cage. This guinea-pig died in four days with typical appearances of plague,

including cervical and submaxillary buboes on both sides. Cultures of *B. pestis* were obtained from its heart-blood.

*House I. 13. 27 (M).*

This house is interesting as being the one furthest from the original focus A which gave evidence of containing plague infection. It immediately adjoins L and is situated within a few yards of the row of houses constituting the southern boundary of the portion of the village known as Koliwada.

The observations and experiments made in it may be arranged in a manner similar to those described in connection with the last house.

*I. An account of the dead rats found in the house.*

On the 17th March a fresh *Mus rattus* was found dead on the floor of an inner room and five fleas were removed from it. The rat showed right and left pelvic buboes and other typical appearances of plague and a culture of *B. pestis* was obtained from the heart-blood. At the same time a mummified *Mus rattus* was found in the same place but was too decomposed to permit of a diagnosis. On the next day a mummified rat was discovered in a loft in an inner room. On the 22nd a dead rat was found in the same room; one flea captured on it was put back in the room. This rat was crawling with maggots but a smear from the liver showed a few typical plague bacilli. A mummified rat was found here at the same time. The search for these rats was suggested partly by the characteristic odour caused by them. On the 24th March a dead mouse was found in front of the house; the organs were somewhat putrid and did not suggest plague infection. On the 27th March a dead adult *Mus rattus* was found in a back room; eight fleas were caught on it but they were put back into the room. This rat proved to be plague infected.

*II. The routine guinea-pig experiment.*

On the 15th February two guinea-pigs were allowed to run free in the house and three days later they were confined in a wire cage. Fleas were observed on the animals on several occasions but they remained healthy for nearly a month. On the 11th of March one of them was found dead; no fleas were seen on it but dissection revealed typical appearances of plague. On the 4th April the other guinea-pig was found to have a bubo and to be sick; it yielded four fleas. After being brought to the laboratory it was killed and found to have a left inguinal bubo and other typical signs of plague.

III. *Special experiments carried out in the house.*

On the 17th March the following animals were put into the house :

(1) Two monkeys, one in a cage with wire gauze and the other in a similar cage but without this protection.

(2) Two guinea-pigs, one in a wire gauze cage and the other in a similar cage without gauze.

(3) Two white rats, one in a tanglefoot cage and the other in a similar cage without tanglefoot.

The animals were kept in the house for five days and were then brought to the laboratory and segregated. No fleas were found on any of these animals.

The unprotected rat died of plague on the 29th March and when examined was found to have double submaxillary buboes with subcutaneous congestion and pleural effusion. As the abdominal organs were somewhat putrid the liver and spleen were inoculated cutaneously into a guinea-pig, which died in four days with typical signs of plague, a culture of *B. pestis* being obtained from the heart-blood and liver.

The other five experimental animals remained healthy. Six fleas were found in the tanglefoot on the cage of the control rat ; three were cat fleas, two were rat fleas, and one was a human flea, but on dissection none were found to contain plague-like bacilli.

*House II. 11. 19 (N).*

This is the last house which calls for special description, but the events which happened in it are somewhat difficult of explanation. The house is situated within a short distance of A and adjoins a building in which a guinea-pig died of plague as early as the 17th February.

On the 12th February two guinea-pigs were put into N. Next day 15 fleas were secured from one of them ; the fleas were brought to the laboratory. Whether the removal of these fleas affected in any way the course of events it is impossible to say, but it is certainly curious that the guinea-pigs and two more which were added on the 14th February remained perfectly healthy till five weeks later.

On the 19th March one of the guinea-pigs was found dead. Examination showed that death was due to plague. Next day the house was searched thoroughly for dead rats but none were found. Two days later a guinea-pig which was in the same cage as the one which was found dead was found to have a submaxillary bubo and to be sick. Only one flea could be obtained from it. The animal was removed to

the laboratory where it died next day. It was found to present the usual appearances of plague.

No fleas were seen on the two remaining guinea-pigs, and although the house was searched thoroughly for dead rats on several occasions none were found.

It is extremely difficult to arrive at any conclusion as to the source of infection of the guinea-pigs which died. There is no evidence that they were infected from plague rats in the house, although it is of course possible that a plague rat may have died among the tiles of the roof and the fleas from it dropped into the room below. The other two guinea-pigs remained healthy till the end of the experiment.

4. *Details concerning the guinea-pigs which died from plague infection received in the houses.* (Table I.)

Out of 51<sup>1</sup> guinea-pigs placed in the houses 36 died of plague (70 %) while 15 survived. Forty<sup>1</sup> guinea-pigs were put in houses which proved to be plague infected and of these only four remained healthy, i.e. 90 % died of plague. Two of these four animals were removed to the laboratory no doubt prematurely with sick companions which subsequently died of plague. The remaining two are those which survived in N.

Of 27 buildings (out of a total of 51 in the village) into which guinea-pigs were put, 21 were proved to be infected by the death of the guinea-pigs from plague: six buildings remained apparently free from infection.

With regard to the number of rat fleas found on the guinea-pigs which died of plague, the results depended entirely on whether the guinea-pig was found to be sick or was found dead. Twelve guinea-pigs were found dead. On 11 of these no fleas were obtained, on the remaining one eight fleas were caught. The largest number of rat fleas caught on a sick guinea-pig was 89, the average number for the sick animals being about 15. We may recall the fact that 24 fleas were caught on the two guinea-pigs which were placed in A. This relatively high count, which, as we shall show later, is characteristic of the infected houses in Koliwada compared with the non-infected houses, strengthens the probability that the dead rat found in A was plague infected. (See vol. VII. p. 445.) It is necessary to point out in this

<sup>1</sup> In this computation no account is taken of guinea-pigs which were lost or were killed by cats, nor do these figures include the guinea-pigs in the special experiments carried out in L and M.



connection that we have had instances of animals escaping infection during a stay in a plague house although fleas obtained on them gave plague when transferred to an animal in the laboratory.

Only twelve cat fleas were captured on all the guinea-pigs placed in the houses although the houses were swarming with cat fleas, as we knew to our cost when we entered them. It is evident, then, that cat fleas have no great liking for the guinea-pig or that they do not remain so long in its fur as the rat flea does.

With reference to the distribution of the buboes in the guinea-pigs dead of plague it will be noted (see Table I) that the overwhelming majority were in the region of the neck. Buboes rarely occurred in the inguinal region and not once in the axilla.

5. *Details concerning the dead rats found in Sion Koliwada.*  
(Maps V and VI.) (Table IV.)

A remarkable feature in our study of plague in this village is the small number of dead rats found. Without doubt at the beginning of the epizootic, partly because some of the houses were occupied and partly on account of the prejudices of the people in the matter of disturbing their household belongings, the search for dead rats was not as thorough as it might have been.

Later when the houses were more under our control and when we were in a position better to appreciate the course of events as regards plague infection in the houses, a very thorough search was made with consequently more satisfactory results.

Still it must be admitted that, considering the severity of the epidemic, the number of plague rats found is very small, although it is amply sufficient to show that an epizootic existed amongst the rats during the period of the experiment. Our experience in this connection affords an excellent illustration of the danger which observers of plague epidemics may incur of concluding that plague rats are absent from an infected locality when a thorough search has not been made.

Again it will be seen from the Table III and Maps V and VI that the course taken by the epizootic corresponds generally both in time and place with that taken by the epidemic amongst the guinea-pigs.

The fact that on very few occasions a plague rat was found before the death of the inmate of the house (whether human or animal) is sufficiently explained by the difficulties met with in the search for rats.



TABLE IV.

*Giving details of rats found in Sion Koliwada, arranged in order of date when found.*

Serial No.	House No.	Date when found	Fleas caught on rat	Bubo	Remarks
1	II. 18. 27 (A)	28. 1. 06	—	—	History of dead rat thrown out.
2	II. 13. 21 (B)	11. 2. 06	—	—	Mummified.
3	II. 25. 37 (E)	17. 2. 06	0	—	Putrid; guinea-pig inoculated cutaneously died of plague.
4	I. 5. 12 (G 1)	20. 2. 06	12 cat fleas 10 rat fleas	Submax.	Cultures from heart-blood, spleen and liver.
5	I. 7. 16 (I)	21. 2. 06	—	—	Mummified.
6	I. 9. 19 (K)	28. 2. 06	12	None	Cultures of <i>B. pestis</i> from heart-blood, spleen and liver.
7	I. 12. 26 (L)	11. 3. 06	0	Right axillary	Putrid; many <i>B. pestis</i> in organs with invol. forms in bubo.
		12. 3. 06	24	Right submax.	Cultures from heart-blood.
		13. 3. 06	0	—	Very maggoty; spleen, a few <i>B. pestis</i> like, many putrefact. organisms.
					Heart-blood, many <i>B. pestis</i> like, many putrefactive organisms.
8	I. 13. 27 (M)	17. 3. 06	5	Retroperitoneal	Culture of <i>B. pestis</i> from heart-blood.
		17. 3. 06	0	—	Mummified.
		18. 3. 06	—	—	—
		22. 3. 06	1	—	—
					—
		22. 3. 06	—	—	—
		24. 3. 06	—	—	—
		27. 3. 06	8	Left axillary	Typ. p.m. subcut. haemorrh. Numerous <i>B. pestis</i> in heart-blood, spleen, bubo.
9	Near II. 1. 1 and 2	24. 3. 06	—	—	Mummified.
10	II. 18. 28 (A)	27. 3. 06	—	—	Skeleton of <i>rattus</i> .

It is not easy to give a full explanation of the reasons for the spread of the epizootic from house to house, though it is certain that the rats in this village have not the slightest difficulty in communicating freely with their neighbours in adjoining houses.

An interesting point in connection with the rat epizootic concerns the length of time the infection persisted amongst the rats. We may conveniently consider this aspect of the epizootic under three headings:

(1) The duration of the infection amongst the rats within the village: if we regard the rat thrown out of A as a plague rat—and we

think this is a justifiable assumption—the length of time between the discovery of this rat and the last plague rat in the village is almost exactly two months.

(2) The rate of diffusion of the infection in the rat population: the infection took at least six weeks to travel from A to M, *i.e.* a distance of only about 300 feet.

(3) The duration of the infection amongst the rats within a house: house M probably gives a fairly correct idea of the length of time infection may persist in the rats in a house, *when no reinfection takes place from outside*. The order of events in the epizootic leading up to the infection of this house is such that reinfection is not likely to have happened. The first plague rat found was on the 17th March and the last on the 27th, that is, the infection persisted in the rats for at least ten days.

A point of considerable importance is that without exception every dead rat found belonged to the species *M. rattus*. It has already been mentioned that no drains or gullies exist in the village. This doubtless accounts for the absence of *M. decumanus* in the village. In the rat collection made throughout the year this species has never once been found.

The buboes in the rats diagnosed as plague were distributed as follows: two submaxillary, two axillary, one pelvic, and in one rat there was no bubo.

6. *Remarks on the houses occupied by men or by guinea-pigs which failed to show evidence of plague infection.* (Map V.)

The most important point to note is that the last three houses in the village, *viz.* those constituting its southern boundary, remained so far as we could discover free from infection. Building I. 14, which closely adjoins the last infected house of all, namely M, in which three rats proved to be plague infected were found, was frequently searched in a very thorough manner but no dead rats were ever found in it. It would seem then that the epizootic had died out in M about 27th March<sup>1</sup>. No other explanation than that it came to a natural end in this house can be brought forward, for there is nothing in the construction of these adjacent houses which differs in any essential respect from that of the plague-infected houses. Two reasons

<sup>1</sup> As will however be seen below, a plague-infected rat was found in Bhandarwada on March 30, 120 feet from house M. (See Map V.)

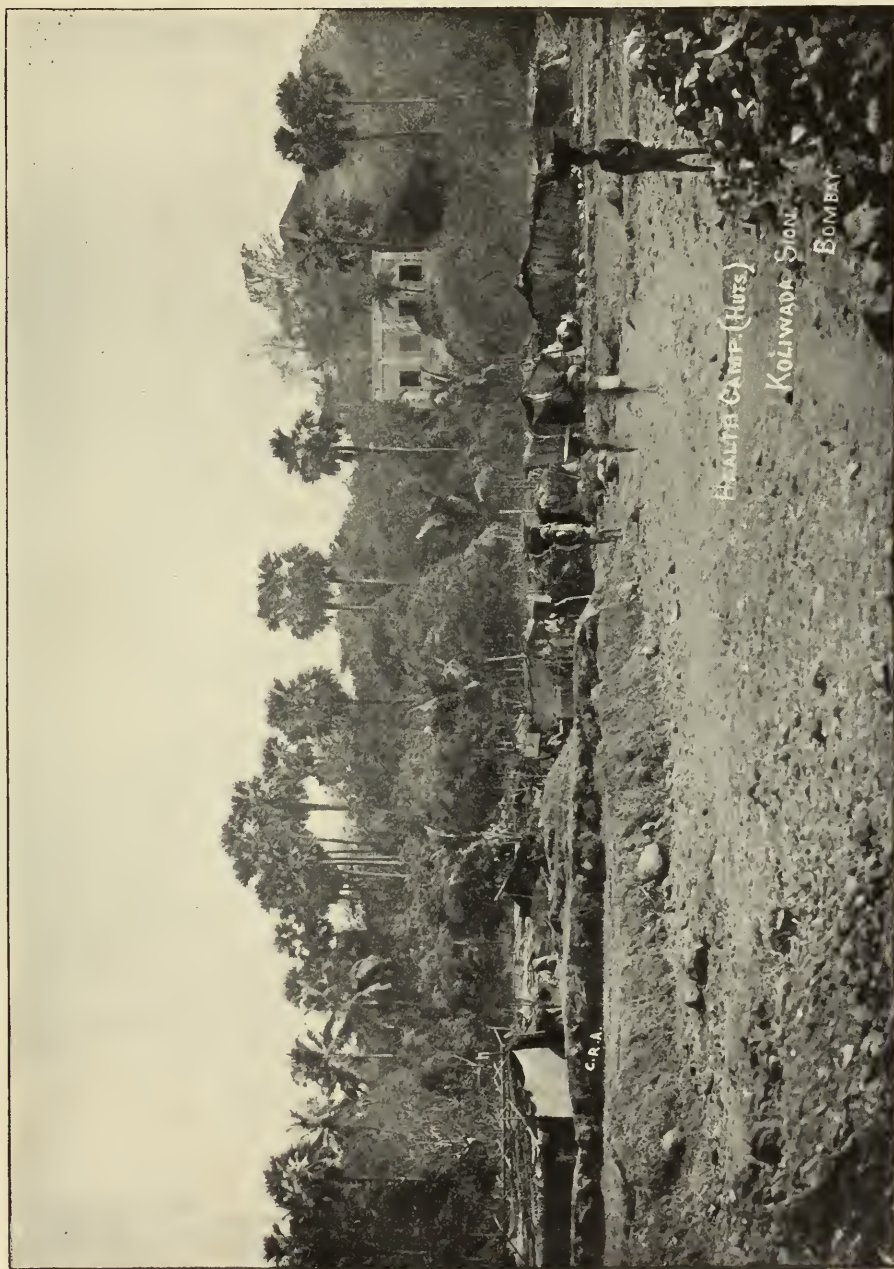
for the cessation of the epizootic at this time (the end of March) may be given. It may be ascribed to an accidental break in its continuity from house to house, infected rats in M failing to come into proximity to the rats in next door. Or the epizootic may have been brought to an end by influences which determine the seasonal prevalence of plague. We believe the latter explanation to be the more probable one, because the epizootic in the City of Bombay about this time began to show marked signs of diminishing.

Besides the houses with guinea-pigs which remained free of plague infection, certain others deserve mention: namely I. 4. 10, II. 10. 17, 18, and II. 20. 31. They were occupied by their inhabitants throughout, and the people, although apparently taking a risk, suffered no harm. In this connection we may state an impression which was forced upon us by our experiences in this village, namely, that the guinea-pig is a more sensitive indicator of plague infection in a house than its human occupants. We are disposed to attribute this to the readiness with which rat fleas live upon guinea-pigs, a question which is dealt with in another paper.

•  
7. *A description of the camp.* (Plates XXXII, XXXIII.)

During the period of the experiment, *i.e.* from the 12th February to the 16th April, the villagers took up their residence in a camp of huts situated only a few hundred yards from the village. Strict observation was kept during this time to ascertain whether any indigenous cases of plague might arise. It is almost certain that none such occurred. One or two cases were indeed found in the camp but these, as we have already pointed out, most probably derived their infection from visits made to infected houses in the village. We had not the slightest reason to suppose that there were any plague rats in the camp. Indeed, so far as we could discover there were no rats in the huts, probably because their construction was so simple and crude that they afforded no shelter for rats. The huts were built of the simplest materials. One, which will serve as an example (Plate XXXIII), was composed of gunny-bag sacking, palm leaves, matting, tatties (bamboo chips), kerosene oil tins, corrugated sheets, planking and bamboo poles.

It only remains to state that the villagers began to break up their camp and to return to the village on the 16th April, a fortnight to three weeks after the discovery of the last plague-infected rat. They acted in this matter, as in their decision to evacuate the village, entirely on



Sion Koliwada: Health Camp, general view.







Sion Koliwada: a hut in Health Camp.



their own initiative, though their choice of this particular day was dictated to a large extent by certain superstitious beliefs. After their return no case of plague occurred in Koliwada.

Before concluding this account of the Koliwada experiment it is necessary to draw attention to its bearing upon the observations made during the earlier period. Briefly, it may be said that the facts related in the foregoing pages seem to us to furnish strong reasons for believing that an epizootic existed in the earlier period and that the dead rat found in A on the 28th January represents the first evidence of plague infection in the village.

Section C. *Observations made in Agriwada<sup>1</sup> and Bhandarwada.*  
(Maps V and VII. Tables III and IV.)

A. *Introductory.* It ought to be pointed out that during the time in which the experiment was progressing in Koliwada the inhabitants of the rest of the village of Sion still occupied their houses. As at one time it appeared probable that the infection might spread from Koliwada into Bhandarwada, on the 21st February a guinea-pig in a wire cage was put into each of four houses in Bhandarwada immediately adjoining Koliwada. Two days later a guinea-pig was put into each of the remaining houses in Bhandarwada, and on the 2nd March this was also done in the case of Agriwada.

We may anticipate so far as to say that very little infection occurred amongst these guinea-pigs. However, a small but interesting outbreak was observed in several houses and this we shall proceed to

TABLE V.

*Table of plague rats found in Agriwada and Bhandarwada.*

Serial No.	House No.	Date when found	Fleas caught on rat	Bubo	Remarks
11	Agriwada 47. 67	23. 2. 06	At least 1 seen	None	Culture of <i>B. pestis</i> from heart-blood and liver.
		5. 3. 06	81	Left axillary	Culture of <i>B. pestis</i> from heart-blood.
		5. 3. 06	12	Left submax.	Culture of <i>B. pestis</i> from heart-blood.
		6. 3. 06	3	Retroperitoneal	Culture of <i>B. pestis</i> from heart-blood and liver.
		8. 3. 06	35	None	Culture of <i>B. pestis</i> from heart-blood and liver.
12	Near 37. 67 Bhandarwada	30. 3. 06	—	Right submax.	Typ. p.m. Very numerous <i>B. pestis</i> in heart-blood, spleen bubo.

<sup>1</sup> Spelt "Agarvada" on map.

describe. The house of principal interest is the one in which the epizootic originated, viz. **47. 67** Agriwada (House P: Map VII).

TABLE VI.

*Table of guinea-pigs which died of plague in Agriwada and Bhandarwada.*

Serial No.	House No.	Date when guinea-pig was put in	Date of death	No. of fleason dead guinea-pig, or before removal to laboratory	Bubo	Remarks
23	Agriwada <b>47. 67</b>	28. 2. 06	5. 3. 06	1	Right and left inguinal and retroperitoneal	—
		28. 2. 06	24. 3. 06	4	Submax.	Chloroformed to death. Chronic plague.
24	Agriwada <b>3165</b>	2. 3. 06	26. 3. 06	6	„	Chloroformed to death. 3 sub-maxillary phlyctenules.
25	Agriwada <b>27. 36</b>	2. 3. 06	1. 4. 06	0	„	—
26	Bhandarwada <b>5. 19</b>	23. 2. 06	2. 4. 06	4	„	Phlyctenule on lowerlip. Chloroformed to death.
27	Bhandarwada <b>30. 55</b>	23. 2. 06	30. 4. 06	0	Right inguinal	—

B. *Events relating to certain plague-infected houses.*

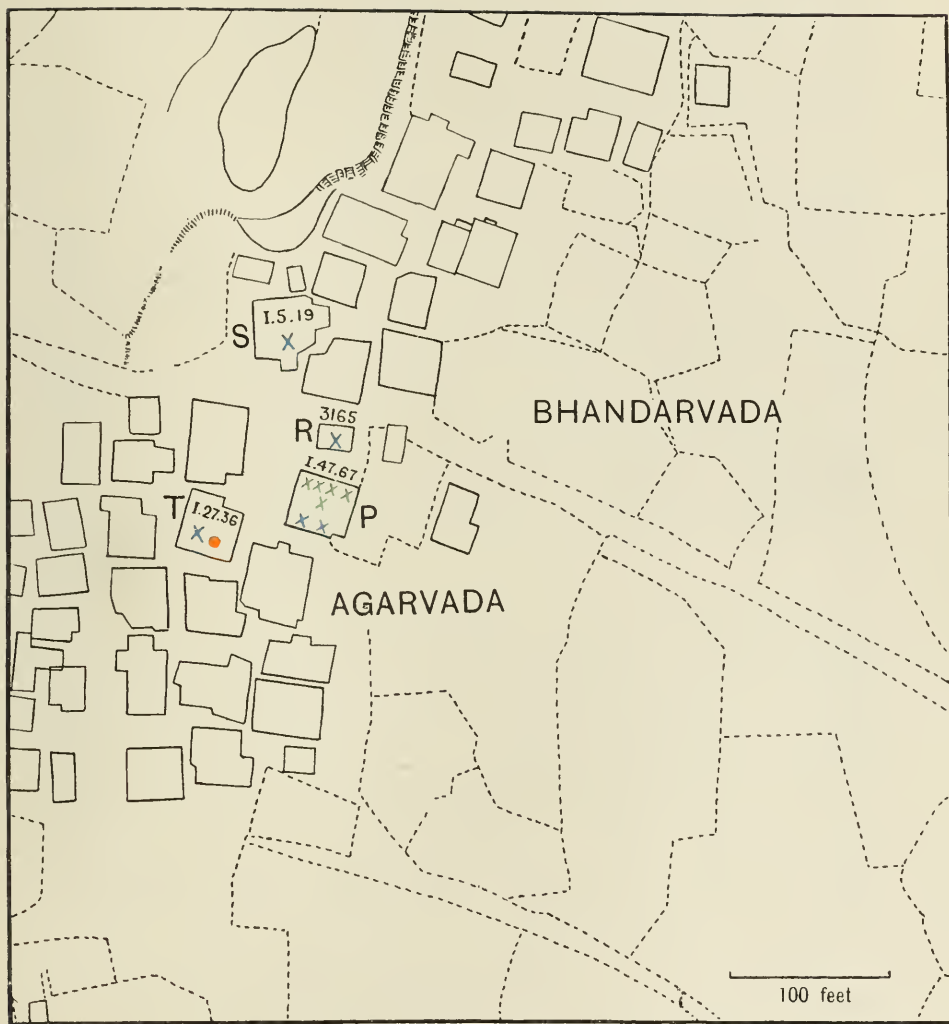
*House 47. 67 (P) Agriwada. (Plate XXXI.)*

On the 23rd February the occupants found a dead rat in the house. It was handed over to us and on examination proved to be plague infected, cultures of *B. pestis* being obtained from the heart-blood and liver. The occupants of the house are vegetable sellers; they buy vegetables in the city, bring them to the house and sell them in the neighbouring districts. We may state at once that we could find no definite clue to the source of the infection.

On the following day the people vacated the house with the exception of an old man, who, however, slept on the verandah outside.

The events of importance which occurred in the house may be conveniently arranged into three groups:

- I. An account of the dead rats found in the house.
- II. The fate of the guinea-pigs placed in ordinary wire cages.
- III. Special experiments.



SION VILLAGE

Human case of plague    Guinea-pig dead of plague    Plague infected rat





I. *Rats.* Mention has already been made of the plague rat (a *Mus rattus*) found on the 23rd February. On the 5th March a dead *Mus rattus* was found in the house: 12 fleas were captured on it and examination proved it to be plague infected, a submaxillary bubo being present and a culture being obtained from the heart-blood. Search revealed a second dead rat lying on the floor in an inner room. This rat yielded 81 fleas. All the fleas were returned to the house. A left axillary bubo was present and a culture of *B. pestis* was obtained from the heart-blood.

Next day a thorough search was made through the whole house, with the result that another dead rat was found in an inner room with part of the head and neck eaten; three fleas were obtained from it. Retroperitoneal buboes were found on post-mortem examination and a culture was obtained from the heart-blood and liver. The house was found to be rat riddled and there was a strong smell of dead rats. Two days later a dead rat was found on the floor of one of the rooms: 35 fleas were caught on it and these were returned to the room. There was no primary bubo but the appearances were typical of plague and cultures were obtained from the heart-blood and liver. A daily search was made for rats subsequently, but none were found. In this house the infection apparently persisted in the rats for 13 days. All the rats found were *Mus rattus*.

II. *The fate of the guinea-pigs.* On the 28th February two guinea-pigs were put into a wire cage in the house. On the 5th March one of them was found dead, and one flea was caught on it. The post-mortem examination revealed typical signs of plague, double inguinal and pelvic buboes being present. On the 19th March the remaining guinea-pig was observed to have a right submaxillary bubo and two rat fleas and two cat fleas were found on it. It was removed to the laboratory where it was chloroformed to death; the post-mortem examination showed that it had a relatively chronic form of plague.

III. *Special experiments.* The following animals were placed in the house in specially constructed cages:

- I. On the 5th March two monkeys, one in a tanglefoot cage the other in a control cage.
- II. On the 5th March two guinea-pigs, one in a wire gauze cage the other in a control cage.
- III. On the 8th March two white rats, one in a tanglefoot cage the other in a control cage.

IV. On the 7th March two guinea-pigs, one in a tanglefoot cage the other in a control cage.

V. On the 9th March two white rats, one in a wire gauze cage the other in a control cage.

I. *Experiment with monkeys.* It has been already stated that 93 fleas were captured from two dead plague-infected rats on the 5th March, and that they were returned to the house. On this date two monkeys were put into the house, one in a tanglefoot cage and the other in a similar cage without tanglefoot. Two days later the tanglefoot cage was examined and 24 fleas (not identified) were obtained on the tanglefoot; 16 of these were dissected but none were found infected. On the 9th both monkeys were examined for fleas; two were found on the non-tanglefoot animal, none on the other. The monkey in the cage without tanglefoot died on the 12th March. A left axillary bubo and abundant pleural effusion were present. Numerous bacilli were seen in the organs, and cultures were obtained from the heart-blood, liver and spleen; the cultures inoculated into rats killed them with typical appearances of plague. The monkey which was protected from fleas by the tanglefoot remained healthy.

II. *Experiment with guinea-pigs.* On the 5th March two guinea-pigs were placed in the house, one in a cage provided with a wire gauze curtain and the other in a similar cage without this protection. On the 9th the animal in the unprotected cage was observed to be sick with a bubo in the neck; three fleas were secured from it. It was brought back to the laboratory and died there shortly afterwards, post-mortem examination revealing typical signs of plague with cervical buboes; a culture of *B. pestis* was obtained from the heart-blood. The animal which was protected from fleas remained healthy.

III. *Experiment with white rats.* On the 8th March two white rats were put into the house, one in a tanglefoot cage and the other in a similar cage without tanglefoot. Next day 16 fleas were obtained on the tanglefoot but none on the rat itself. On the 10th two fleas were got on the rat in the tanglefoot cage, but it must be noted that the tanglefoot paper was only 2 to 2½ inches broad. Next day both rats were removed from the house and kept isolated. The rat in the tanglefoot cage remained healthy. On the control rat before removal 13 fleas were captured, four of these being cat fleas. This rat was found dead on the 13th March. Right inguinal and right axillary buboes were present, numerous plague bacilli were present in the spleen and bubo, and cultures of *B. pestis* were obtained from the heart-

blood and liver. The 15 fleas which were obtained from the two rats before removal from the house were transferred to a guinea-pig in a flea-proof cage but the animal remained healthy.

IV. *Second experiment with guinea-pigs.* On the 7th March two guinea-pigs were put into the house, one in a cage with tanglefoot and the other without this. The details of this experiment need not be given since both guinea-pigs remained healthy.

V. *Second experiment with white rats.* On the 9th March two white rats were put into the house, one in a cage protected with fine meshed wire gauze and the other in a similar cage without gauze. Both animals remained healthy.

*House R 3165 Agriwada.*

As will be seen from the map (VII) this is a small house closely adjoining the last. On the 16th March it was found to be vacated. The woman who occupied it admitted that she found a dead rat on the floor and that she threw it out. Although this house was thoroughly searched for rats on several occasions none were found.

However, on the 24th March the guinea-pig which had been placed in the house was observed to be sick. Next day three small phlyctenules were noticed on the right side of the neck and a bubo was felt here. The phlyctenules resembled vaccination vesicles, each being surrounded by a zone of redness and being situated in the centre of a small hairless patch of skin; they were exactly like the vesicles seen when plague material is rubbed into the skin of a guinea-pig or like those seen occasionally on guinea-pigs experimentally infected by fleas. Six fleas were removed from the guinea-pig which was then brought back to the laboratory, where next day it was chloroformed to death, post-mortem examination showing submaxillary buboes and other typical appearances of plague.

*House 5. 19 (S) Bhandarwada.*

This house is situated only a few yards from the last. On the 2nd April the guinea-pig in the house was found to be sick with a phlyctenule on the lower lip and a right submaxillary bubo; four fleas were found on it. It was removed to the laboratory where it was chloroformed to death, examination revealing submaxillary and cervical buboes and other signs of plague. No rats were found on search in the house. The occupants vacated the house for about a week and then returned to it.

*House 27. 36 (T) Agriwada.*

This house closely adjoins house P. On the 1st April the guinea-pig in the house was found dead. No fleas were recovered from it but on examination it proved to be plague infected.

Next day a plague case in this house was reported to us. The patient was a young man, 17 years old and by occupation a bookbinder in Bombay City. He stated that he came home from work on the evening of the 31st March feeling ill and feverish. The case was undoubtedly one of plague, a right femoral bubo being present. The patient died on the 6th March and the house was then vacated. It was searched thoroughly for dead rats but none were found. The question of the source of infection in this case is a difficult one. There was a clear history of dead rats in the office in which the man worked, and in fact about the same time a case of plague in the adjoining village of Wadhala came to our notice, in which the source of infection appeared to be the same office. An alternative explanation is that the case we are considering became infected from rats in the house which in turn owed their infection to the epizootic in house P. This view naturally receives support from the circumstance that the guinea-pig in the house died of plague, as we have described.

There can be no doubt that a limited epizootic had broken out in this quarter of Agriwada. It may at first sight seem strange that considering the severity of the infection in house P the outbreak did not extend further than it did. According to our view the most feasible explanation is the one we have already given when discussing the cessation of the epizootic in Koliwada, namely, that influences determining the seasonal decline of the epizootic had begun to take effect.

Two incidents remain to be noticed which happened in Bhandarwada.

(1) On the 30th March a rat was captured near **37. 67**, 120 feet south-west of house M in Koliwada. (See Map V.) It proved to be plague infected, a right submaxillary bubo being present. As this rat was caught only three days after a plague rat was found in house M Koliwada it may be that it represents the last trace of the Koliwada epizootic.

(2) The second occurrence is that on 30th April a guinea-pig was found dead in a house (marked O in Map V) 100 feet further south. It was found to have died of plague. The occupants continued to live in the house but nothing further happened and we could obtain no



clue as to the source of infection of this animal. This is the last event which pointed to the presence of plague infection in the village. As the guinea-pigs in all the other houses remained healthy they were removed to the laboratory on the 30th May.

Section D. *General survey of plague in Sion Village.*

It has been explained that our original intention was to make as complete a study as possible of the outbreak of rat and human plague which was expected to occur in the village of Sion, giving particular attention to the origin of the epizootic and of the epidemic.

We have described how our efforts in this direction were not as successful as we hoped, on account of the concealment of the epizootic at the commencement and of the first human cases. The available evidence, however, inclines strongly to show that the infection in Koliwada was not of indigenous origin but was imported, as it were by a mere chance, from Bombay City, having being brought by a woman who resided in an infected quarter of the city.

It is impossible to state exactly in what form the infection was brought to the village. With our present knowledge we can recognise only two media capable of conveying infection from one place to another, namely, either infected rats or infected fleas. In the case of Koliwada the latter is the more probable explanation of the two. It is obvious, however, that infection transported by infected rat fleas must remain, except under experimental conditions, incapable of proof.

Whatever may have been its origin an epizootic undoubtedly broke out amongst the rats inhabiting the houses, the infection spreading gradually amongst them throughout the village.

Taking advantage of the voluntary evacuation of the village (Koliwada) by its inhabitants, it was decided to substitute in as many of the houses as possible a guinea-pig population. As the subsequent course of events showed this procedure proved ideal for studying the problem of the relation of the epizootic to the infectivity of houses. The epidemic was entirely under our control for purposes of observation—much more so than any human epidemic could ever be. Thus it was possible frequently to note the number of fleas on the guinea-pigs and to make a complete diagnosis of every guinea-pig which died. Moreover, the outstanding advantage of the scheme was that the guinea-pigs in separate buildings were kept isolated from each other, so that there

could be no question in any instance of infection having been conveyed by direct contact with a sick animal.

It has been shown that corresponding to the epizootic amongst the rats many of the houses became infective, the proof of this being that in the early period of the epizootic several human cases occurred and that at a later period most of the guinea-pigs which were placed in the houses died of plague.

*The association of the epizootic with the epidemic.* It is apparent from a review of the facts that the guinea-pig epidemic in Koliwada was closely related to the outbreak amongst the rats. Thus, it has been shown that the "epidemic" followed a fairly definite course, extending gradually from the houses in the vicinity of the original focus, till it reached the two houses near the southern limit of this quarter of the village, which were proved to be badly plague infected. The rat epizootic was roughly coextensive with the epidemic, and coincided with it in point of time. Moreover, in the two houses mentioned and in 47. 67 (P) Agriwada the number of plague rats found corresponded to the degree of infectivity of the houses, as judged by the success of the experiments carried out in them. Again, in several instances the experimental animals in the houses remained healthy until plague-infected rats were found. The fact that plague rats were not found first in all the houses is doubtless due to difficulties to which we have already alluded.

*The mode of infection of the human cases and the guinea-pigs.* With regard to the mode of infection of the human cases and of the guinea-pigs, it is certain that the source of infection was common to all.

The possibility of direct infection, *e.g.* by infected excreta from a human case in one building to a case in the other or from a guinea-pig in one building to a guinea-pig in another, is absolutely excluded. In the first instance the human occupants of the infected buildings had no relations with each other on account of differences in caste, and in the case of the guinea-pigs it is evident that such a mode of infection was rendered impossible by their isolation in the houses.

The hypothesis that the common source of infection was a soil infection is in the highest degree improbable. We have proved experimentally that nothing short of gross contamination of soil with plague bacilli is capable of causing infection to guinea-pigs and that only a small percentage of guinea-pigs exposed to such infection develop plague.

It may be noted, also, that the distribution of the buboes in the

guinea-pigs infected experimentally in this way does not correspond with the distribution in the guinea-pigs which received their infection in the houses of Koliwada. Moreover, the animals which became infected in the cages of special construction were protected from any possible risk of soil infection.

We may, now, gather together the facts which are in favour of the view that the infection was brought about by the intermediary of infected rat fleas:

(a) All the houses which proved to be infective contained rat fleas, and rat fleas in considerable numbers were readily captured on the guinea-pigs. An interesting point in this connection is that during the period when the houses remained free of infection, and in houses which escaped infection altogether, the guinea-pigs harboured much fewer fleas than when infection was present in the house. Thus, the average number of fleas caught on sick guinea-pigs was nearly 15 per animal, whereas on healthy guinea-pigs it was rare to find more than three or four fleas. This difference cannot be explained altogether by the fact that fleas especially attack sick animals, because on a few guinea-pigs, which were observed to be sick and which died from causes other than plague, very few fleas were obtained when the house was free from plague infection. The reason for the larger number on the animals in the infective houses is simply that the fleas readily attack the guinea-pig after the death of their proper host.

(b) On one occasion fleas with bacilli in their stomachs, morphologically resembling *B. pestis*, were obtained in a house in which plague rats were found and in which experimental animals died of plague.

(c) On one occasion fleas removed from a plague rat found in a house in which two guinea-pigs died of plague gave plague to a guinea-pig in the laboratory when placed with it in a flea-proof cage.

(d) In certain experiments monkeys, guinea-pigs and white rats in cages of special construction were introduced into houses in which plague rats were found. The animals which were confined in cages designed so as to protect them from fleas remained healthy, while several control animals in cages similar in construction, but allowing entrance of fleas, died of plague.

(e) On a few of the guinea-pigs small cutaneous phlyctenules were observed in the region where the bubo was situated. These phlyctenules were identical with those which have been seen when infected fleas were fed for experimental purposes on the skin of guinea-pigs.

(f) A point of the greatest importance is the distribution of the buboes in the guinea-pigs. A table (VII) has been constructed to show the regional distribution of the buboes, (1) in the guinea-pigs infected in the Sion houses and (2) in guinea-pigs infected experimentally by fleas in flea-proof cages. The correspondence between the two tables is extremely striking. While this is so the buboes in the Koliwada guinea-pigs do not correspond in point of distribution with those produced by any other known method of infection. It may be added that mesenteric buboes were never found in any of the animals.

TABLE VII.

*Comparison of the distribution of the primary bubo in guinea-pigs (a) infected in the houses in Sion and (b) infected experimentally by fleas.*

		Sion guinea-pigs	Infected experimentally by fleas
No bubo	... ..	2.3 %	0.9 %
Single buboes	... ..	93	82.4
Neck	... ..	92.5	88.8
Groin	... ..	7.5	11.2
Axilla	... ..	0	0
Multiple buboes	... ..	4.6	16.7
Neck glands involved	... ..	100	100
Of total cases with buboes the neck glands affected	... ..	92.8	90.6

*Note* :—The only serious discrepancy in the above table is the relatively large proportion of guinea-pigs with multiple buboes amongst those infected experimentally by fleas. This may doubtless be explained by the fact of great concentration of infection in the case of the guinea-pigs in this series.

The above evidence appears to us so convincing that we feel justified in attributing the mode of infection of the guinea-pigs in the infected houses to the agency of infected rat fleas.

At this point reference may be made to the mode of infection of the rats which died of plague during the epizootic in Koliwada and Agriwada. Although the number obtained is small yet it is sufficient to show that the distribution of the buboes corresponds to what has been proved to exist in several thousand plague rats obtained during the epizootic in Bombay City and also to the distribution in rats experimentally infected by fleas in flea-proof cages. We conclude, therefore, that the rat flea was again the transmitting agent of infection in the Sion epizootic.

*Noteworthy points arising out of the investigation.* Several points of interest which emerge from our study of plague in Sion may be recalled :



(1) It seems worth while drawing attention to certain difficulties which were met with in making these epidemiological investigations. In the first place, we experienced great difficulty in tracing the origin of the epizootic. In similar circumstances it will probably be always impossible to obtain any definite evidence of the medium by which infection is imported. In the second place, an illustration has been furnished of the difficulty of deciding between two alternatives when investigating the source of infection of a human case. Thus the patient in **27. 36** (T) Agriwada may have been infected at work or he may have received his infection from plague rats in the house.

(2) Although there was a relatively widespread infection of the buildings in Koliwada—45% of the total buildings in the village were proved to be infected—yet there is not the slightest evidence for the view that infection by direct contact with a sick animal played any part in the spread of the infection. This possibility was, indeed, excluded by the nature of the experiment.

(3) Considering the severity of the epidemic the number of plague rats found seems very small, notwithstanding that during the latter part of the observations a very thorough and extensive search was made, short of breaking up the houses, *i.e.* digging up floors, removing tiles, etc. Our experience in this respect points to the danger of concluding that plague rats are absent from an infected locality, unless a thorough search is carried out.

(4) The facts relating to the persistence of infection amongst the rats may be summarised thus:

(a) The infection persisted amongst the rats in the village for two months.

(b) The rate of spread of the epizootic is indicated in the statement that the infection in the rats took six weeks to travel 300 feet.

(c) In the case of two houses, in which it is improbable that reinfection took place, the infection lingered amongst the house rats in one case for at least 10 days, and in the other for 13 days.

*Final conclusions.* The principal conclusions at which we have arrived after a careful consideration of all the facts of the Sion observations may now be stated. They appear to us to have an important bearing upon the epidemiology of human plague.

(i) The accidental introduction of plague infection, on a single occasion only, into a hitherto uninfected locality led to a widespread epizootic amongst the rats living in the houses.



(ii) Corresponding both in time and in place to the epizootic there was a dissemination of an infecting agent within the houses.

(iii) The infecting agent within the houses which was the cause of the guinea-pig epidemic took the form of infected rat fleas.

(iv) The Koliwada experiment furnishes no evidence that the infection, although widespread, was conveyed in any single instance from one experimental animal to another by direct contagion; on the contrary, the conditions under which the experiment was carried out entirely precluded the possibility of direct infection from animal to animal.

(v) The severity of the epidemic amongst the guinea-pigs was due solely to the accessibility of the animals to rat fleas from infected rats. It cannot with any show of reason be directly associated with insanitary conditions which may have obtained either within or outside the buildings.

### III. OBSERVATIONS IN WADHALA VILLAGE. (Map VIII.)

Wadhala is a village with about 1500 inhabitants and is situated  $1\frac{1}{2}$  miles to the south of Sion.

The observations made in this village are very unsatisfactory, chiefly because the villagers shortly after the first indigenous cases of plague occurred evacuated the greater part of the village and went to live in a camp about a mile distant. For this reason the keys of the houses could not readily be obtained, so that it was difficult to gain frequent entrance into them to search for dead rats. As a result the number of rats from the village, which were proved to be plague infected, is very small although there is evidence that an epizootic existed.

The following notes may, however, be recorded, since they serve to illustrate certain points of interest.

The first two cases of plague in the village were found in adjoining buildings on the western side on 3rd March. In both instances there was a definite history of residence in quarters of Bombay City which we knew to be plague infected. One of the patients (1) was brought to Wadhala during his illness, while the other (2) was attacked with the disease on the day after arrival. We could obtain no evidence of rat mortality in either of the buildings. Two guinea-pigs were, however, put into each of the houses on 11th March; no fleas were caught

MAP VIII

WADALA

24









on any of the guinea-pigs before removal, and all of them remained healthy. We could discover no evidence of extension of infection from the human cases either amongst rats or men. These cases, therefore, appear to us to be noteworthy as showing that persons coming from an infected district to an uninfected locality, although themselves incubating the disease or suffering from it, do not necessarily introduce the infection into the new locality.

The first case of plague in the village which we had reason to think was indigenous, was that of a boy (3) on the south side who was attacked on 25th March. This case offers an example of the difficulty of arriving at a conclusion as to the source of infection even when a careful investigation has been made. The boy was attacked with plague on 25th March and died in two days. We were informed that a dead rat was found in the house on the day before he was attacked; a putrid rat was actually found in the house on 11th April. On 2nd April two guinea-pigs were put into the house. One of these was found dead of plague on 6th April; the other, on which no fleas were caught, was removed at the same time but remained healthy. There can be no doubt, then, that infection was present in the house. On inquiry, however, we found that the boy attended a school just outside the boundary of the village. In this school a dead rat was said to have been picked up on 13th March and another on 15th March. In consequence the school was closed for a week. The school re-opened on 19th March and as already mentioned the boy was attacked on 25th March. Guinea-pigs placed in the school rooms did not contract the disease. It is thus possible, either, that the boy received his infection in the school, or, that he carried infection from the school to the house, the house-rats becoming infected and afterwards transmitting the infection to himself, but dead rats were found close by on 16th March and before on 23rd February elsewhere. On 28th March, a man, Govind Rama (4), living in a house close by, was attacked with plague. This man lived and worked in an infected quarter of Bombay, but occasionally visited this house which belonged to his father. We were informed later that two dead rats had been found in this house about 16th March and that they had been thrown away by this man. A neighbour informed us further that a dead rat was found in the house early in February. In this building also a man (5) was attacked with plague on 30th March, and a sister-in-law (6) of Govind Rama who lived with him was attacked on 14th April and died in two days. She had been living in the camp for 12 days before her illness.

Two other plague cases occurred in buildings not far removed from the house of Case 3. The first (7) is that of a young man attacked on 1st April who died in three days. The other is the case of a man (8) who was attacked on 11th April.

From the map it will be seen that the cases we have just noted occurred within a limited area. A review of the data relating to the presence of dead rats in the houses makes it very probable that the outbreak was associated with an epizootic amongst the house-rats in this area.

As already mentioned the villagers soon after the first cases of plague were found evacuated the greater part of the village and occupied a camp about a mile distant. After remaining in the camp for about two months the villagers returned. Several plague cases occurred shortly afterwards towards the centre of the village. In one or two instances there was a history of finding dead rats in the houses, but only one was obtained by us which was proved to be plague infected. This rat was found on 27th May and an occupant (9) of the house was attacked with plague on the same day. It appeared to us, therefore, that an epizootic existed in this part of the village and that the plague cases were the result of the premature return of the villagers to their houses.

In conclusion we may refer to two interesting cases of rat plague which were unaccompanied by human cases.

(1) A dead rat was found outside a house (A) on 23rd February. It had a submaxillary bubo and other typical signs of plague. It was, therefore, the first event which pointed to indigenous plague infection in the village. And yet we could obtain no evidence of an extension of infection either amongst the rats in this house and the neighbouring houses or amongst the villagers living in the vicinity. The question of the source of infection of this rat is a difficult one. It is a suggestive fact that three coolies working for the Commission lived in this house. All of these men assisted in various ways in the work connected with the daily examination at the laboratory of rats from Bombay City. It is possible, then, that they carried infected rat fleas on their persons and that by this means infection was conveyed to the rat. Again, it may be mentioned that two of the coolies assisted in the investigations we were carrying out in Sion Koliwada, which at this time was badly plague infected.

(2) A rat, caught alive on 18th August on the eastern side (B), was found to be infected with acute plague. Two guinea-pigs were put

into the house but no fleas were taken on them and they remained healthy. We could obtain no evidence as to the source of infection of this rat. No sign of infection, either amongst rats or man, was discovered in the village during  $2\frac{1}{2}$  months previous to this date.

#### IV. OBSERVATIONS IN PAREL VILLAGE. (Map IX.)

In the introduction to the epidemiological studies made in four Bombay villages it was pointed out that these villages had been selected because they occupied isolated positions and because the inhabitants, of three of them at least, followed an employment which kept them confined for the most part to their villages and to the tract of country immediately surrounding them. Parel, the observations in which we are about to describe, differs from the other three villages inasmuch as, although fairly isolated, it has very intimate connection with various parts of the city through a large number of the inhabitants who work there during the day and sleep in the village at night.

Moreover, Parel, in addition to the difference in the nature of the employment of its inhabitants, differs from the other three villages in the following respects:—

(1) The construction of the houses much more closely approaches that found in the city than is the case in the other villages.

(2) The village is furnished with a drainage system, *i.e.* sewers have been constructed along the main streets. Only a few of the buildings, however, are directly connected with this sewage system. None of the other villages possess any sewage system.

##### 1. *General description of the village; its situation, the structure and population of the buildings.*

Parel village is situated almost in the centre of Bombay Island. It forms one of the suburbs of the city and is distant from the Fort about five miles. The village itself is isolated from the rest of the city. It is surrounded on all sides by considerable stretches of more or less open country, or rather the large compounds of residential and other buildings. For example, along the west and north sides it is separated from the extensive compound of the Old Government House by a high stone wall. The southern portion is bounded by a road (Parel Back Road), which separates it from the compound of the Veterinary college and hospital (the Bai Sakarbai Dinshaw Petit Hospital for Animals). Along the

eastern border runs the Parel Tank Road, which separates the village from Parel Hill, a sparsely populated residential quarter of Bombay.

Some details regarding the number of buildings, houses and inhabitants found in each block and in the whole village are given in Table VIII; it will be seen that the population of the village is 3525; that it contains 150 buildings, which are divided into 862 tenements or houses; and that the average number of houses in each building is nearly six. The number of houses in each building, however, varies considerably. The average number of inhabitants per building is 23·5, and the average number of inhabitants in each house is four. Blocks II and III show the largest number of inhabitants per house, but it is to be noted that these blocks contain the majority of the houses with more than one room and are occupied by the better class inhabitants of the village.

TABLE VIII.

*Showing number of Buildings, Houses and Inhabitants in Parel Village.*

Block no.	No. of buildings	No. of houses	No. of inhabitants	Average no. of houses per building	Average no. of inhabitants per building	Average no. of inhabitants per house
I	31	140	614	4·5	19·8	4·4
II	10	29	140	2·9	14·0	4·8
III	15	68	339	4·5	22·6	5·0
IV	15	103	457	6·9	30·5	4·4
V	14	157	556	12·6	39·6	3·8
VI	20	137	546	6·8	27·3	4·0
VII	45	228	873	5	19·4	3·8
Total	150	862	3525	5·7	23·5	4·1

The construction of the buildings in the village very closely resembles that found in the city. They are, for the most part, flimsily constructed, some being merely huts consisting of a wooden framework covered with palmyra palm leaves, or of bamboo laths besmeared with earth and cowdung. Others are made with brick and mortar or stones bound together with clay. Very few are built with stone and lime. The majority of the buildings are roofed with "country" tiles, but a few are covered with "Mangalore" tiles. The floors of the houses, for the most part, are made of earth, rammed down and covered with cowdung, but in some buildings cement floors are found. Nine characteristic Bombay "chawls" are scattered throughout the village. These chawls are large buildings, of one or more stories, divided up into single room tenements or houses. A number (about 22) of ruined buildings are scattered throughout the village.



In addition to the dwelling houses many native shops are to be found. These are chiefly occupied by petty grocers and grain dealers and are situated along the main village street, marked "Parel Village Road" in the map. There are also four shops which sell firewood and one or two licensed liquor shops. These shops have been mentioned because of their marked infestation with rats. A few goldsmiths and tailors ply their trade in the village. There is a small market where fish, meat, and vegetables are sold in the mornings.

A stable for buffaloes is also situated in the village. These animals supply milk to some of the villagers. The majority of the poorer classes, however, namely those living in one-roomed houses, derive their milk supply from goats, which, with hens, cats and dogs, at night share their common apartment and during the day move about the verandahs and land adjoining the houses. Here the animals find their food supply, which, in part at least, consists of the leavings of the people's meals, carelessly thrown out from their dwellings. This method of disposal of refuse is the common one adopted in the village. The scavenging is in the largest part effected by the above mentioned domestic animals, aided by numerous kites, crows and rats.

These remarks on the structure of the buildings and the habits of the people prepare us to expect a considerable rat infestation, which subject we now pass on to consider.

## *2. Observations on the rat infestation of the village.*

A very thorough and systematic examination of the rat infestation was made during the period the village was kept under observation. This was effected by daily setting a number of traps in the different houses in rotation according to the census numbering. In certain houses, however, the tenants refused to take rat-traps because of their religious scruples, while others, after the traps had been set in their houses on a few occasions only, objected to be further troubled with them. The indifference of the majority of the inhabitants to the presence of rats in their dwellings was very noticeable. A few traps were set in places outside dwelling houses, but in these traps *Mus rattus* was seldom taken.

Table IX gives the details of the rats caught alive and found dead in weekly periods from the 20th November 1905 to 1st November 1906, when the rat catching operations were brought to a close. The Table shows that 2195 *Mus rattus* were captured during the year. This number



TABLE IX.

*Showing, for weekly periods, number of rats trapped alive and found dead in Parel Village.*

Week ending	Alive					Dead					Plague infected
	<i>rattus</i>	<i>decumanus</i>	<i>Nesokia</i>	Mice	Musk rats	<i>rattus</i>	<i>decumanus</i>	<i>Nesokia</i>	Mice	Musk rats	
20th to 26th Nov. '05	164	0	0	5	36	0	0	0	0	0	Nil
27th Nov. to 3rd Dec. '05	116	0	0	6	13	0	0	0	0	0	"
4th to 10th Dec. '05	125	0	0	6	25	0	0	0	0	0	"
11th to 17th "	178	1	0	2	15	0	0	0	0	0	"
18th to 24th "	115	1	0	11	22	0	0	0	0	0	"
25th to 31st "	128	0	0	5	21	0	0	0	0	0	"
1st to 7th Jan. '06	87	0	0	14	10	0	0	0	0	0	"
8th to 14th "	73	0	0	3	2	0	0	0	0	0	"
15th to 21st "	60	0	0	2	4	4	0	0	0	0	"
22nd to 28th "	70	0	0	13	7	2	0	0	0	0	"
29th Jan. to 4th Feb. '06	42	0	0	1	0	0	0	0	0	0	"
5th to 11th Feb. '06	37	1	0	5	4	0	0	0	0	0	"
12th to 18th "	30	0	0	7	3	0	0	0	0	0	"
19th to 25th "	27	0	0	1	0	1	0	0	0	0	"
26th Feb. to 4th March '06	48	0	0	2	0	0	0	0	0	0	"
5th to 11th March '06	21	0	0	1	0	0	0	0	0	0	"
12th to 18th "	29	0	0	4	1	1	0	0	0	0	1 <i>rattus</i>
19th to 25th "	50	0	0	0	3	0	0	0	0	0	Nil
26th March to 1st April '06	44	0	0	0	5	1	0	0	0	0	1 <i>rattus</i>
2nd to 8th April '06	12	0	0	1	1	2	0	0	0	0	2 "
9th to 15th "	32	0	0	0	1	0	0	0	0	0	Nil
16th to 22nd "	65	0	0	0	3	1	0	0	0	0	"
23rd to 29th "	13	0	0	0	0	1	0	0	0	0	"
30th April to 6th May '06	21	0	0	0	0	0	1	0	0	0	"
7th to 13th May '06	17	0	0	0	2	2	0	0	0	0	1 <i>rattus</i>
14th to 20th "	24	0	0	0	2	0	0	0	0	0	Nil
21st to 27th "	25	0	0	0	1	4	1	0	0	0	1 <i>decumanus</i>
28th May to 3rd June '06	11	0	0	0	0	2	1	0	0	0	1 <i>rattus</i>
4th to 10th June '06	9	0	0	0	0	0	0	0	0	0	Nil
11th to 17th "	13	0	0	0	0	0	0	0	0	0	"
18th to 24th "	16	0	0	0	0	0	0	0	0	0	"
25th June to 1st July '06	25	0	0	0	1	1	1	0	0	0	"
2nd to 8th July '06	26	0	0	2	0	1	0	0	0	0	"
9th to 15th "	9	0	0	0	0	2	1	0	0	0	"
16th to 22nd "	26	0	0	0	2	0	0	0	0	0	"
23rd to 29th "	12	0	0	1	0	0	0	1	0	0	"
29th July to 5th Aug. '06	5	0	0	0	0	0	0	0	0	0	"
6th to 12th Aug. '06	16	0	0	0	0	1	0	0	0	0	"
13th to 19th "	31	0	0	0	1	0	0	0	0	0	"
20th to 26th "	22	0	0	0	1	0	0	0	0	0	"
27th Aug. to 2nd Sept. '06	15	0	0	0	0	0	0	0	0	0	"
3rd to 9th Sept. '06	31	0	0	0	0	0	0	0	0	0	"
10th to 16th "	37	0	0	1	1	0	0	0	0	0	"
17th to 23rd "	42	0	0	0	3	0	0	0	0	0	"
24th to 30th "	29	0	0	0	0	0	0	0	0	0	"
1st to 7th Oct. '06	50	0	0	0	0	1	0	0	0	0	"
8th to 14th "	44	0	0	1	1	0	0	0	0	0	"
15th to 21st "	10	0	0	1	0	0	0	0	0	0	"
22nd to 28th "	38	0	3	5	1	1	0	0	0	0	"
29th Oct. to 1st Nov. '06	25	0	0	3	1	1	0	0	0	0	"
Total	2195	3	3	103	193	29	5	1	0	0	6 <i>rattus</i> 1 <i>decumanus</i>

gives an average of nearly 15 rats per building in the whole village, or  $2\frac{1}{2}$  per house. If, however, only the buildings in which a reasonable number of traps had been set are counted, the average number of rats works out at 22 per building.

Table X shows the number of rats caught each fortnight, together with the number of traps set. From these data the number of rats caught each fortnight per 100 traps set has been calculated and is recorded in column 3. The approximate number of times the village had been trapped is stated in column 4 of the Table. A study of this Table shows that, while during the first round of trapping 65 rats were taken per 100 traps set, by the third round this number had been reduced to 49, by the fifth round to 38, by the seventh round to 25, and so on, until on the thirteenth round only 10 rats were taken per 100 traps

TABLE X.

*Table showing, for fortnightly periods, the progress of rat catching operations in Parel Village.*

Date	No. of <i>rattus</i> trapped alive	No. of traps set	No. of <i>rattus</i> per 100 traps set	No. of times the village was trapped
20th Nov. to 3rd Dec. '05	280	432	64.8	$1\frac{1}{2}$
4th to 17th Dec. '05	303	624	48.6	3
18th to 31st „	243	647	37.6	5
1st to 14th Jan. '06	160	640	25.0	$7\frac{1}{2}$
15th to 28th „	130	669	19.4	10
29th Jan. to 11th Feb. '06	79	510	15.5	$11\frac{1}{2}$
12th to 25th Feb. '06	57	573	9.9	13
26th Feb. to 11th March '06	69	484	14.3	$14\frac{1}{2}$
12th to 25th March '06	79	607	13.0	$15\frac{1}{2}$
26th March to 8th April '06	56	441	12.7	17
9th to 22nd April '06	97	400	24.2	18
23rd April to 6th May '06	34	347	9.8	19
7th to 20th May '06	41	377	10.9	$20\frac{1}{2}$
21st May to 3rd June '06	36	291	12.4	$21\frac{1}{2}$
4th to 17th June '06	22	251	8.8	22
18th June to 1st July '06	41	252	16.3	$22\frac{1}{2}$
2nd to 15th July '06	35	216	16.2	23
16th to 29th „	38	252	15.1	$23\frac{1}{2}$
30th July to 12th August '06	21	149	14.1	24
13th to 26th August '06	53	143	37.0	$24\frac{1}{2}$
27th August to 9th Sept. '06	46	158	29.1	25
10th to 23rd Sept. '06	79	230	34.3	$25\frac{1}{2}$
24th Sept. to 7th Oct. '06	79	250	31.6	26
8th to 21st Oct. '06	54	162	33.3	$26\frac{1}{2}$
22nd Oct. to 1st Nov. '06	63	186	33.9	27
Total	2195			

set. This latter number is calculated on the number of rats caught during the fortnightly period 12th to 25th February.

After this date the number of rats taken remained at a comparatively low figure till the middle of August, when during the 24th round of trapping it rose to 37 per trap set, and thereafter was maintained at about this figure, till the rat catching operations were suspended. During the twenty-seventh and last round of trapping 34 rats per 100 traps set were taken, a number which is more than half the number taken at the first round. It is to be remembered that by this time over 2000 rats had been removed from the village.

We can conclude, then, that the rat infestation of Parel is very considerable and that, despite the capture and removal from the village during the year's operations of a large number of rats, equivalent to nearly two-thirds of the human population, the number at the end of the operations was apparently not very greatly diminished. The rats removed were evidently rapidly replaced by young ones, the effect of this being most marked during the month of August, when in Bombay the fecundity of *Mus rattus* is greatest (see above, p. 749).

TABLE XI.

*Showing rat infestation and its relation to population in each block in Parel Village. Only for buildings in which at least 27 traps had been set.*

Block No.	Rats caught	Traps set	Rats caught per 100 traps set	Population of trapped buildings	No. of buildings trapped	Average population of trapped buildings
I	457	1466	31	495	18	28
II	85	343	25	107	8	13
III	398	954	42	267	10	26
IV	184	1257	15	396	11	36
V	305	1339	23	600	13	46
VI	468	1663	28	533	19	28
VII	180	1511	13	667	17	39
Total	2077	8533	24	3065	96	32

We can now study the rat infestation of the village in greater detail and for this purpose we would refer the reader to Tables XI and XII. In these Tables only the buildings in which at least one or more traps were set during each round of trapping the village are included. In Table XI the figures for each block, in XII for each building, are recorded. From Table XI it will be seen that blocks IV, V and VI, although they contained a large population or a large average number of inhabitants per building, yet yielded, when compared with the other less populous

blocks, a smaller number of rats. When we consider individual buildings, as displayed in Table XII, we find that the population of a building has no direct relation to the number of rats it is possible to capture in it. We also observe that rat infestation of buildings is very irregular, some containing many rats, others none or a few only.

TABLE XII.

*Block I.*

Building No.	No. of rats	No. of traps	Rats per 100 traps	Population
3	16	42	38	47
4	51	148	35	67
5	21	106	24	38
7	28	50	56	7
8	49	113	38	24
10	14	55	25	28
11	88	110	80	7
12	103	196	53	67
13	11	57	19	18
15	5	87	7	16
16	6	97	6	27
17	0	96	0	27
19	17	47	36	21
21	16	98	16	21
23	1	27	4	27
25	19	35	54	7
26	1	41	2	35
29	11	61	18	12
Total	457	1466	31	496

Average rats per 100 traps :—31.

Average inhabitants per building :—28.

*Block II.*

1	19	66	29	13
2	2	22	9	5
3	25	79	32	19
5	11	29	38	2
6	10	55	18	10
7	7	22	32	15
8	7	42	16	12
9	4	28	14	31
Total	85	343	25	107

Average rats per 100 traps :—25.

Average inhabitants per building :—13.

*Plague in Parel Village**Block III.*

Building No.	No. of rats	No. of traps	Rats per 100 traps	Population
1	0	84	0	13
4	14	47	30	26
7	301	393	76	70
9	10	50	20	20
10	13	25	52	18
11	19	149	13	31
12	8	40	20	11
13	13	51	25	11
14	11	61	18	28
15	9	54	16	36
Total	398	954	42	267

Average rats per 100 traps :—42.

Average inhabitants per building :—26.

*Block IV.*

1	22	138	16	37
2	8	162	5	34
3	19	169	11	19
4	17	137	12	54
5	24	127	19	70
6	21	105	20	69
7	35	102	34	23
8	18	126	14	36
9	7	83	8	7
10	10	85	12	11
11	3	23	13	36
Total	184	1257	15	396

Average rats per 100 traps :—15.

Average inhabitants per building :—36.

*Block V.*

1	20	220	9	56
2	8	165	5	45
3	10	47	21	26
4	10	46	21	26
6	9	33	27	2
7	2	31	6	1
8	34	88	39	40
9	28	33	84	8
10	161	382	42	138
11	6	111	5	119
12	11	88	13	31
13	3	50	6	59
14	3	45	6	49
Total	305	1339	23	600

Average rats per 100 traps :—23.

Average inhabitants per building :—46.



*Block VI.*

Building No.	No. of rats	No. of traps	Rats per 100 traps	Population
1	4	113	4	53
2	30	145	21	27
3	70	60	117	12
4	20	46	43	3
5	13	105	12	5
6	32	128	25	25
7	36	74	48	16
8	9	28	32	11
9	9	83	11	19
10	13	41	32	14
11	38	102	37	35
12	2	89	2	37
14	5	55	9	35
15	21	39	60	3
16	21	109	19	26
17	11	108	10	30
18	12	78	15	41
19	56	109	41	40
20	66	155	43	101
Total	468	1667	28	533

Average rats per 100 traps :—28.

Average inhabitants per building :—23.

*Block VII.*

1	3	82	4	15
6	29	98	30	31
7	4	133	3	21
8	4	107	4	35
9	27	256	11	101
10	18	202	9	108
11	48	62	77	55
12	5	56	9	26
16	4	45	9	8
17	7	27	26	5
31	1	32	3	33
32	3	54	6	53
33	7	113	6	47
36	15	111	13	83
42	3	40	8	20
43	2	51	4	16
44	0	42	0	10
Total	180	1511	13	667

Average rats per 100 traps :—13.

Average inhabitants per building :—39.

We would now draw attention to a few of the worst rat infested buildings and consider what features they had in common, which might offer an attraction to rats. Details regarding five buildings of this description are given in Table XIII.

TABLE XIII.

*Details of badly rat infested buildings in Parel Village.*

Serial No.	Block No.	Building No.	Rats caught	Traps set	Rats per 100 traps	No. of tenements in building	Population of building
1	VI	3	70	60	117	5	12
2	V	9	28	33	84	3	8
3	I	11	88	110	80	2	7
4	VII	11	48	62	77	17	55
5	III	7	301	393	70	16	70

The first building on the list is an old one, with ground-floor and upper story. The walls are made of bricks covered with plaster and the roof of country tiles. The building is divided into five tenements and contains twelve inhabitants. Bags, containing grain and ground nuts, had been stored on the ground-floor verandah for some months. Further, there is a loft in which an accumulation of rubbish had been collected. Seventy rats were caught in sixty traps set in this building, giving a take of 117 rats per 100 traps set. The second building is a small ground-floor one with a roof of country tiles. The floor is of rammed mud and the walls of stone jointed with clay. They are both riddled with rat holes. It is divided into three tenements and inhabited by eight people. One room, the largest, in the centre of the building is used as a grain merchant's shop, where grain of all kinds is stored in bags. In this building 28 rats were caught in 33 traps set. The third building very closely resembles the second in structure. It is divided into two tenements, both of which are used for petty grocer's and grain merchant's shops, as well as for the living rooms of the seven inhabitants. The floor and walls are riddled with rat holes. Eighty rats were captured in 110 traps set. The fourth building very closely resembles the first in structure, being an old building, which has evidently seen better days. It consists of a ground-floor and upper story. It has now been divided up into seventeen tenements occupied by 55 inhabitants. In this house 48 rats were caught in 62 traps. The fifth building is in some ways the most noteworthy, inasmuch as it yielded the largest number of rats, namely, 301 caught in 393 traps. It also is an old building, consisting of ground-floor and two upper stories, the topmost deserving the name of a loft rather than a story.

In structure it resembles the other buildings mentioned above. On the ground-floor in front there are two small grocer's shops. The floor of these shops is made of mud and is riddled with rat holes. The top story or loft is divided into a number of rooms by wooden partitions. The population of this building, numbering 70, is distributed among 16 tenements.

These five rat infested buildings are all very old, the walls and floors being riddled with rat holes. The majority possess ample attraction for rats in the grain shops on the ground-floors. Further, rats are afforded shelter in the country tiles of the roofs, as well as in the soft floors and crumbling walls. It is noteworthy that these buildings include all the grocers' shops in the village in which we were allowed to set traps. The remaining shops of this type, in which we were not allowed to set traps because of the religious scruples of the owners, when examined, gave ample indication of being rat infested. It would appear, then, that the presence of such shops in a building markedly increases the rat infestation.

TABLE XIV.

*Details of rat free (?) buildings in Parel Village.*

Serial No.	Block No.	Building No.	Rats caught	Traps set	No. of tenements	Population
1	I	17	0	96	6	27
2	III	1	0	84	3	13
3	VII	44	0	42	3	10

In the whole village only three buildings, which had been properly trapped, showed a complete absence of rats. The details of these buildings are given in Table XIV. One of them, namely, the second on the list, can for the following reasons hardly be said to be rat free. The building has a ground-floor and upper story. The ground-floor is used as a godown, in which a large quantity of rice is stored. The upper story is divided into three single room tenements. It was in these rooms that traps were set, none being placed in the rice godown below. An examination of the godown showed rat holes and other evidence of the presence of rats. It is possible that rats were attracted by the abundant food supply to the godown rather than to the rooms above in which the traps were set. Of the other two rat free buildings we can give no very satisfactory explanation, other than that they were occupied by very poor people, who brought their food supply in daily and probably left little or nothing, after partaking of their meals, which could support rats.

We may now briefly refer to certain other points connected with the rats in the village. (1) Twenty-nine *Mus rattus* were picked up dead in the village, six of these dead rats being proved to be infected with plague. The remaining number, twenty-three, was all that we were able to collect, which might be said to represent the normal apparent mortality among this species of rat. This number must evidently fall far short of the real normal mortality. In order to further investigate this point we opened up some rat holes and burrows and exposed skeletons of rats in them. It would appear, therefore, that many rats must die in places which are not readily accessible to man. (2) It is interesting to note the presence of *Mus decumanus* in this village, probably associated with the sewage system. Three rats of this species were trapped alive and five were found dead, one of these latter being proved to be plague infected. (3) Three *Nesokia bengalensis* were trapped alive and one was found dead. (4) Mention need only be made of a number of mice, 103, which were trapped alive. This number, however, gives no adequate idea of the proportion of mice to rats in the village, as the traps used were adapted for rats and not for mice, the latter being easily able to escape. One of the houses which was free from rats, viz. the first on the list in Table XIV, was infested with mice.

### 3. Remarks on plague among the rats in the village.

We now pass on to consider the presence of plague among the rats in the village. Although 1321 rats caught alive were examined, up till the week ending 11th March not one among that number was found infected with plague, either in the acute or chronic form. The whole village by this time had been trapped fourteen times. We were, therefore, fairly assured of the absence of plague among the rats at this date.

However, the possible infection of the rats of the village from a northerly direction was brought home to us by the discovery of plague-infected rats in some godowns in the compound of Old Government House on the 10th and 11th of January 1906 (*vide* Map IX). We have already pointed out that this compound is separated from the village by a high stone wall and it is interesting to note, that although the village is very adjacent to these godowns the infected rats seem to have been kept out of the village for some months by this wall. Part of this infected rat colony appears to have migrated in a westerly direction, along the wall surrounding the village, for a plague-infected animal was

## MAP IX

### PAREL VILLAGE

Showing plague cases and plague  
infected rats



P A R E L T A N K



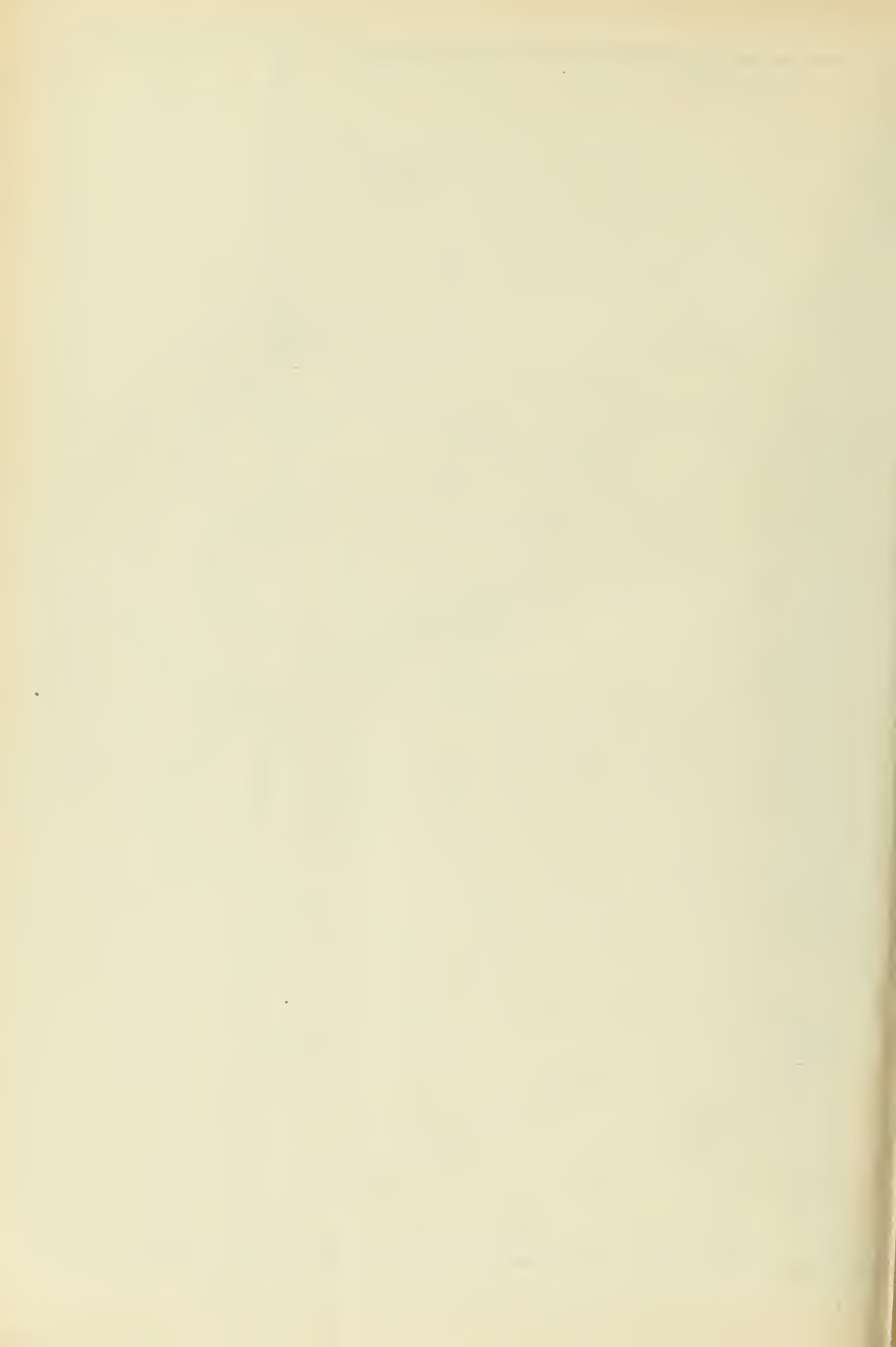


**PAREL VILLAGE**  
 Showing plague cases and plague infected rats

Scale 100 feet to five-eighths of an inch

- Human plague attack (serial number)
- Plague infected *M. rattus*

758



found in this direction, still within Government House compound, more than a month later, namely, on the 17th February. A strict watch was kept for the appearance of plague among the rats of the village along this northern boundary, but no evidence of an epizootic was obtained till the 17th of March, when a dead plague-infected rat was found to the east in Block V, building 1. Adjacent to this building an opening, in the form of an open doorway, exists in the wall separating Government House compound from the village: this is indicated by a cross in the Map. It seems highly probable that the epizootic in the village was started by an extension through this opening of the epizootic in Government House compound. A second plague-infected *rattus* was found in the same building on the 29th March. This was followed by the discovery of a plague-infected rat on 3rd April in the compound of building No. 11, Block IV, adjacent to a latrine, marked L in Map IX. This latrine was used by the inhabitants of building 11. A fourth plague-infected *rattus* was found on the 5th April, lying on the ground at the corner of building 1, Block V. Whether the rat had died here or been thrown out of the house we could not ascertain. As will be seen from the map all these rats were found in the same neighbourhood.

No more plague-infected rats were found in this neighbourhood after the 5th April and the epizootic here had apparently come to an end. Why this should have been the case we can only speculate, but it is noteworthy that in the week embraced between the 1st and 7th April there was a marked fall in the number of plague-infected *rattus* obtained throughout Bombay. It is also worthy of note that the rat infestation of the houses immediately adjoining building 1, Block V, namely, buildings 2, 3, and 4 of this block and 10 and 11 of Block IV, was slightly below the average for the houses in these blocks (*vide* Table XII).

It remains now to refer to three plague-infected rats found in the village in the month of May. The date and position where these rats were found are shown in the Map. The first rat was found on the 9th and the second on the 24th May. This latter rat belonged to the species *Mus decumanus*, while the former was a *Mus rattus*. The third rat, also a *Mus rattus*, was found on the 28th May. In connection with these rats it is worth noting that during this month the number of imported human cases of plague was very large. The epidemic, as far as the village was concerned, was apparently at its height, while the epizootic had rapidly declined. It is possible that these rats acquired the disease from infected rat fleas imported by man. That the epizootic did not extend from these rats might be due to the fact that the conditions

TABLE XV.

Showing Plague Cases in Parel Village.

Serial no.	Names	Block	Building	House	Age	Sex	Occupation	Dates of			
								Attack	Death	Putting in guinea-pigs	Taking out
1	Ládkí Wallabhji	VI	1	16	16	F	Household work	18. 3. 06	22. 3. 06	22. 3. 06	23. 3.
2	Laxmíbai Shivá	V	10	84	18	F	„ „	21. 3. 06	25. 3. 06	30. 3. 06	7. 4.
3	Súndrábái Náráyán	IV	11	89	11	F	Nil	3. 4. 06	8. 4. 06	9. 4. 06	12. 4.
4	Bárki Dinánáth	IV	11	92	30	F	Household work	4. 4. 06	11. 4. 06	9. 4. 06	12. 4.
5	Ranchod Niláji	IV	11	92	60	M	Fitter G.I.P. Ry.	3. 4. 06	12. 4. 06	—	—
6	Báloo Shivá	V	12	135	18	M	Cooly	6. 4. 06	7. 4. 06	—	—
7	Bhimá Vishnoo	V	1	12	30	F	Household work	13. 4. 06	Recovered	—	—
8	Báboo Báloo	V	1	1	6	M	Nil	13. 4. 06	15. 4. 06	—	—
9	Gangábái Rámá	I	26	118	18	F	Mill hand	20. 4. 06	24. 4. 06	24. 4. 06	1. 5.
10	Mooktábai Yessoo	VI	Market Room	40	F	Vegetable Seller	21. 4. 06	24. 4. 06	23. 4. 06	25. 4.	
11	Govardhan Mátádin	I	10	53	23	M	Cooly G. I. P. Ry.	22. 4. 06	Recovered	23. 4. 06	25. 4.
12	Bábáji Krishná	VI	9	41	70	M	Goldsmith	22. 4. 06	24. 4. 06	24. 4. 06	28. 4.
13	Jayerám Atmárám	VII	11	129	20	M	Carpenter	25. 4. 06	Recovered	—	—
14	Náráyan Ráoji	VI	20	134	45	M	Mill hand	30. 4. 06	2. 5. 06	2. 5. 06	8. 5.
15	Krishná Rámá	III	7	16	26	M	„	3. 5. 06	5. 5. 06	5. 5. 06	15. 5.
16	Haribá Ráoji	VII	—	—	20	M	„	5. 5. 06	11. 5. 06	12. 5. 06	17. 5.
17	Ratin Bhámbiá	V	1	8	35	F	Sweeper	9. 5. 06	11. 5. 06	12. 5. 06	17. 5.
18	Santoo Ránoo	VI	20	134	35	M	Mill hand	9. 5. 06	13. 5. 06	12. 5. 06	17. 5.
19	Ganoo Mánkoo	I	5	28	25	M	„	10. 5. 06	15. 5. 06	12. 5. 06	17. 5.
20	Párvati Bhágyá	V	11	133	18	F	Household work	10. 5. 06	19. 5. 06	20. 5. 06	31. 5.
21	Shivrám B. Parelkar	IV	14	96	20	M	Clerk B.B.L.	12. 5. 06	Recovered	—	—
22	Jánoo Vithoo	V	10	79	25	M	Mill hand	14. 5. 06	22. 5. 06	23. 5. 06	31. 5.
23	Gangáram Ratnoo	V	1	4	22	M	Cooly P. W. Dept.	19. 5. 06	21. 5. 06	19. 5. 06	23. 5.
24	Bhágoo Vithoo	VII	12	134	30	F	Mill hand	25. 5. 06	Recovered	25. 5. 06	31. 5.
25	Báloo Vithoo (son of 24)	VII	12	134	6	M	Nil	25. 5. 06	Recovered	25. 5. 06	31. 5.
26	Bháoo Lingoo	I	5	28	30	M	Barber	27. 5. 06	Recovered	29. 5. 06	4. 6.
27	Vaná Himat	V	10	64	25	M	Moneylender's clerk	16. 7. 06	20. 7. 06	—	—
28	Samná Doolá	V	10	74	28	M	Grain Merchant	27. 7. 06	31. 7. 06	2. 8. 06	6. 8.





at this time in Bombay were evidently very unfavourable for its propagation, as will be seen from the rapid decline in the number of plague-infected rats received from the city at the laboratory during this month (*vide supra*).

4. *Plague among the inhabitants, and its relation to rat plague.*

We pass on now to consider plague among the inhabitants of the village. Table XV gives the details of the cases, arranged in serial order according to the date of the attack. They appear on Map IX as red spots.

In arriving at a diagnosis of the disease it was considered expedient not to attempt to take cultures, as any endeavour in this direction, we felt sure, would lead to the concealment of cases and frustrate our object in obtaining as early information as possible of the occurrence of the disease among the villagers. We had, therefore, to rely on purely clinical methods. In only one case, however, had we any doubt as to the cause of death, namely, case 2. This case was not seen by us during life, but was certified as a plague death by the district registrar.

From Table XV it will be seen that 28 cases of plague occurred in the village, giving an attack rate of nearly 8 per 1000 inhabitants. Twenty-one of these cases ended fatally, giving a case mortality rate of 75 %. Table XVI gives the number of deaths each month which occurred in the village from plague, cholera and other causes. From this table it will be seen that throughout the year 23 % of total deaths in the village were due to plague.

Human plague began in Parel on March 21st with a case (2) on the east side of the village. There was no bubo and the diagnosis was doubtful; no source of infection could be definitely determined. The next day, a case (1) was brought from Bombay and died the same day in the south-west corner of the place. These were followed by a series of five cases (3, 4, 5, 7, 8) in the north-east corner, falling ill between April 4th and April 13th and definitely associated in time and place with the epizootic which was found to prevail in the same group of houses between March 17th and April 5th. The remaining 21 cases were not associated with any ascertained rat plague in Parel. Nine of them (13, 14, 15, 16, 17, 18, 19, 24 and 25) were imported already infected at various dates between 25th April and 25th May: seven (6, 9, 11, 12, 20, 21 and 22) were probably infected outside the village as they had recently visited places where rat plague was known to be present: while the

source of the infection in the remaining five could not be determined. Though one of the imported cases infected the house in which she died, as shown by the capture of plague-infected fleas therein, none of them gave origin to any epizootic.

TABLE XVI.

*Showing the number of deaths from Plague and other causes for each month in Parel village.*

Months	Plague	Cholera	Other causes	Total
November '05	0	0	3	3
December '05	0	0	3	3
January '06	0	0	5	5
February '06	0	0	3	3
March '06	2	0	6	8
April '06	9	0	1	10
May '06	8	7	6	21
June '06	0	1	5	6
July '06	2	2	8	12
August '06	0	2	6	8
September '06	0	0	6	6
October '06	0	0	5	5
Total	21	12	57	90

We may now proceed to the details of these cases, grouping them in four groups according to the adjudged mode of infection.

*Group I. Cases in which the infection could be attributed to the epizootic in the Village.*

Only five cases (3, 4, 5, 7 and 8) could be definitely attributed to the presence of the epizootic in the village.

The first three cases probably derived their infection from the epizootic, represented by the rat found in the compound of the building in which they lived (Building 11, Block IV). This rat was found on the morning of the 3rd March lying dead near a latrine which was used by the inmates of this building. Although the three patients were related to one another, Nos. 3 and 4 lived in one set of rooms, while No. 2 lived in another adjacent set of rooms. In none of the rooms had dead rats been found.

Cases 7 and 8 occurred in building 1, Block V. Their infection might reasonably be attributed to the presence of plague among the rats in this building, as evidenced by the finding of dead plague-infected rats in or near this building on the 17th and 29th March and 5th April. Both cases were attacked on the 13th April. They occupied

different rooms in the building. Case 8 lived with three other persons in a single room on the 1st story of the building. Case 7 lived with his mother and another child in a room on the ground-floor. In neither of these rooms had dead rats been found.

None of the five cases, so far as we could learn, had been in direct contact with plague-infected rats; nor had dead rats been found in any of the rooms occupied by them.

The day after the death of the patient and six days after her attack two guinea-pigs were placed in the rooms occupied by case 3. On examination after three days only two fleas were taken. The guinea-pigs remained healthy.

Group II. *Cases in which the infection could not be attributed to the village, some of the patients (a) having been employed in a place which was proved to be infective, while others (b) had been in contact with dead rats somewhere outside the village.*

(a) This group includes cases 14, 15, 16, 18, 19, 24, and 25. They were all employed at a cotton spinning mill in the Sewri section of Bombay. Dead rats had been found in several parts of this mill, but especially in the mixing department, where cases Nos. 14, 15, 18 and 19 worked. Two of these men had thrown out the rats. Cases 16 and 24 were employed in other parts of the same mill. Case 24 lived with her son (25) who occasionally visited the mill with his mother. The woman had noticed dead rats in the godown where she worked. All these cases, and three others investigated by us who lived in other parts of Bombay but worked in this mill, were attacked between the 30th April and 10th May.

An interesting experiment was done in the mixing room in the mill. On the 12th May two guinea-pigs were placed in it; they were examined for fleas on the 14th May. On one guinea-pig 108 and on the other 150 rat fleas were captured. After the fleas had been removed the guinea-pigs were taken to the laboratory and isolated. They remained healthy. The fleas were transferred to a fresh guinea-pig confined in a flea proof cage in the laboratory. This guinea-pig died of plague on the 20th May. The mixing room was thus proved to be infective.

It is important to note that these cases lived in different parts of Parel village and that we could obtain no evidence of a rat epizootic at their homes which did not prove infective to guinea-pigs. The infection therefore may reasonably be attributed to the mill in which they worked.



(b) *Cases 1, 13 and 17 are included in this group.*

*Case 1.* This woman was brought to the village when moribund. She lived with her family in a house in Samuel Street in Mandvi district of Bombay City. Dead rats had been found in this house a few days before she, with other three members of her family, was attacked with the disease. Her friends believing that a change of air would do her good, hired a room in Block V building 1 in Parel village and brought her there on the 21st March. She died on the evening of her arrival. Her friends, after removing the body, quitted the house, leaving behind them a certain amount of kit and bedding. Two guinea-pigs were placed in the room on the 22nd March. These animals were examined for fleas on the 23rd, four rat fleas being taken, three on one and one on the other. The guinea-pigs were isolated in the laboratory. The fleas were not dissected. The guinea-pig on which the three fleas were found died of plague. As will be seen from the map we had at this time no evidence of the presence of plague among the rats in this part of the village. Bearing in mind this fact and the experiments recorded in a previous report, which showed that animals did not contract the disease in contact with the sick, if fleas were excluded, it is more than probable that the guinea-pig was infected by rat fleas imported to the village by the patient or her friends.

*Case 13.* This man was employed as a carpenter in the Chukla section of Bombay City. Three days before he was attacked he had noticed dead rats in his workshop.

*Case 17* of this group only remains to be detailed. She had been living in the building, Block V, building 1, in which plague-infected rats had been found a month previous to her attack. She left her house to nurse her daughter in another part of the city. Dead rats had been found in this latter house. She returned to Parel after the death of her daughter, herself at this time suffering from plague, and died there on the 11th May 1906.

It remains to be stated that guinea-pigs were placed in the rooms in Parel occupied by cases 14, 15, 16, 17, 18, 19, 24 and 25. Cases 14 and 18 and cases 24 and 25 lived in the same rooms. Guinea-pigs were put into the room occupied by cases 14 and 18 on two occasions, namely, on the 2nd May immediately after the death of case 14, and two on the 12th May the day before the death of case 18. In all the rooms tested, except in those occupied by cases 15 and 16, no rat fleas were captured on the guinea-pigs, which all remained healthy. In the room occupied by case 16 only one flea was trapped, while in the



room occupied by case 15 twenty-seven fleas were captured. It should be noted that this latter room was situated in one of the most notoriously rat infested buildings in the village, namely, building 7, Block III, in which 301 rats had been captured during the year's operations.

This group of cases which presumably received their infection outside the village constitutes 36 % of the total plague cases which occurred in the village. As will be seen when we consider the next group of cases, there is good reason to believe that some other cases also acquired their infection outside the village.

*Group III. Cases which might have acquired their infection by visiting places, in which epizootic plague was known to be present about the time they were attacked.*

Seven cases, namely, 6, 9, 11, 12, 20, 21 and 22, fall into this group. Two of these cases, namely, 6 and 21, might have been placed with some reason in group II. These men had visited the laboratory, where an extensive examination of plague-infected rats was being carried on. *Case 6* had come in search of employment in connection with these operations and had stood on one or two occasions near the place where the dead rats were prepared for examination. The patient had not been inoculated. *Case 21* had been employed in the laboratory for some time; he had, like the rest of the staff of the laboratory, been inoculated with Haffkine's vaccine. The room where he worked was adjacent to the verandah where the rats were examined.

*Case 9* was employed in a mill in Bombay where several other of the workers had been attacked with plague. The patient herself had not noticed or heard that dead rats had been found in the mill. Several plague-infected rats had, however, been found in the neighbourhood of the mill before this patient was attacked. The same remarks apply to *case 11* who was employed in a railway workshop near the above mentioned mill.

*Case 12* occasionally visited the district in which the above mentioned mill and workshop are situated. He had not seen dead rats in any place he visited, nor had he been in any house where he was aware that plague cases had occurred.

*Case 20* was employed as a daily household servant in a building close to the mill in Sewri district, where cases 14, 15, 16, 17, 18, 19, 24 and 25 probably derived their infection. She had not personally noticed dead rats at the place of her work.

*Case 22* was employed in another mill, adjoining the above mentioned one in Sewri district. He had not noticed or heard of dead rats being found in this mill.

Two guinea-pigs were placed in each of the rooms occupied by the cases of this group, except in the rooms occupied by cases 6 and 21, which cases, as we have seen, probably contracted the disease at the laboratory. The number of fleas caught on the guinea-pigs exposed in these rooms is recorded in Table XV. Not one of the guinea-pigs contracted plague.

From the remarks made above we have fair grounds for concluding that the cases of this group acquired their infection outside the village. If we add these seven cases to the ten cases of group II we can calculate that 17 or 61% of the total plague cases acquired their infection outside the village.

Group IV. *Cases in which the source of infection was undetermined.*

In this group we have cases 2, 10, 23, 26, 27 and 28. *Case 2* was a suspicious case of plague. She died after suffering from fever without bubo for two days, the disease being certified as plague by the district registrar. She was employed in a mill in the Sewri district of Bombay, but had not been to work for more than a month. She occupied herself in household duties and was in the habit of carrying her husband's midday meal to his place of work at the Great Indian Peninsular Railway Workshop at Byculla. She had not noticed dead rats at any place she had visited. No dead rats were found in her house nor, as far as we were aware, at her husband's workshop. Two guinea-pigs placed in the room in which she lived with her husband and brother-in-law remained healthy, and only two rat fleas were found on the animals.

*Case 10*: a female, who had lived with her husband in a small hut near the market, where they sold vegetables and groceries. She had been in the habit of visiting various parts of the city for the purpose of buying these commodities. One cat flea was captured on the two guinea-pigs placed in this hut, the animals remaining healthy after being isolated in the laboratory.

*Case 23* lived in a building (Block V, building 1), in which plague-infected rats had been found more than a month previous to the date on which he was attacked. He was employed in various parts of the city in connection with the work of the Public Works Department. Two guinea-pigs placed in his house, a single room, on the day he was attacked trapped only one rat flea. The guinea-pigs remained healthy.

*Case 27* is a similar case to the above. This man had come to Bombay in search of employment, for which purpose he wandered about and slept in various parts of the city. Three days before he was attacked he had found employment in Parel village.

*Case 26* presents some features of interest. He lived in the same house as case 19. He was a barber by occupation and in this capacity visited all parts of the village, but, however, seldom went outside it. He was attacked twelve days after the death of case 19. Two guinea-pigs, which had been placed in the house during the illness of case 19 and removed from the room ten days before the present patient was attacked, remained healthy. Moreover, two fresh guinea-pigs placed in the room two days after this latter patient was attacked also remained healthy. We, therefore, consider that it is probable that this patient acquired his infection outside the house he lived in and quite apart from case 19. It is interesting to note that a dead plague-infected *Mus decumanus* was found on the 24th May, that is three days before this man was attacked, near building 8, Block I, on a vacant piece of ground, not far from the patient's house and in the direct line of a short cut between his house and the main road of the village.

*Case 28* is also noteworthy. He had lived for some time in the village and had not recently left it even to go to the city. He was employed in a grain merchant's shop and had to handle the grain which was frequently imported from Bombay. No infected rats had been found for some time in the village and no dead rats had been found in or near his house throughout the year the village was under observation. Parts of the city however, even at the season when the patient was attacked, contained plague-infected rats. The source of his infection can only be conjectured, but it seems possible that he might have been infected in connection with the handling of the grain in the shop where he was employed.

In reviewing the whole series of 28 cases of plague which occurred in this village, it is worthy of note that only in four instances did two or more cases occur in the same house. These cases are:

Cases 4 and 5 in house 92 building 11 Block IV.

Cases 19 and 26 in house 28 building 5 Block I.

Cases 14 and 18 in house 134 building 20 Block VI.

Cases 24 and 25 in house 134 building 12 Block VII.

The first two cases (4 and 5) occurred in the area in which a plague

epizootic was present. They were attacked almost simultaneously. From what has been said it seems unlikely that cases 19 and 26 acquired their infection in the house in which they lived. Cases 14 and 18 both probably derived their infection in the mixing room of the mill at which they worked and not in the house in which they lived. Cases 24 and 25 appear to have contracted their infection in the godown of the mill where the mother, case 24, worked.

In all the houses where plague cases occurred a number of individuals were living in the same room as the sick. In no instance did we get any evidence to show that the sick communicated the disease to their healthy attendants and friends.

#### *Conclusions.*

The main conclusions to be derived from this epidemiological study may be summarised under the following heads:

(1) The structure of the buildings and the habits of the people living in them favour a high degree of rat infestation.

(2) The rat infestation of the buildings has no direct relation to the number of inhabitants living in them.

(3) Although a large number of rats, equivalent to nearly two-thirds of the human population, was removed from the village during the year's operations, still a large number remained, the loss evidently being rapidly made good by reproduction.

(4) The plague epizootic in the village was of a very limited character. This might be due, either to the late date in the epizootic period when the disease started, or, in part perhaps, to a considerable temporary reduction in the rat population. New foci of rat infection were possibly started through imported infection; but the disease at this time made no progress among the rats.

(5) From the available evidence it appears that 17 of the cases (61%) acquired their infection outside the village.

(6) There is no evidence to show that the disease spread from man to man when infection was imported into the village by sick persons. Persons living in the same room as the sick did not contract the disease.



V. OBSERVATIONS IN THE VILLAGE OF WORLI.  
(Map X with Plate XXXIV.)

As in the other three villages on Bombay Island in which epidemiological observations were conducted, so in Worli elaborate preparations were made for the study of the plague epidemic and epizootic, should the disease break out. We had every reason to expect an epidemic of plague in this village, for it had been smitten by the disease each year for the past nine years. However, as far as plague is concerned, only three events of importance occurred during the year in which the village was under observation.

Worli village is situated on a narrow peninsula on the north-western shore of Bombay Island. The inhabitants are nearly all fishermen. The buildings, or rather huts, inhabited by these fisher-folk are, for the most part, constructed of rough stones held together by clay or some poor quality of lime. The roof of the huts is generally covered with palmyra palm leaves. A few more substantial buildings are to be found in the village, but the Bombay chawl is unknown. Indeed, the whole features of this village closely resemble those found in any Konkan village, and markedly contrast with the conditions found in Bombay City or those described as existing in Parel village. In connection with the census operations, some details of which are given in Table XVII, we divided the village into four blocks. It will be seen that the population of the village is 2508 and that the inhabitants live in 593 houses, situated in 388 separate buildings. These figures give an average of four inhabitants per house and a little over six per building.

TABLE XVII.

*Showing Houses, Buildings and Population of Worli Village.*

Block No.	Building No.	Houses	Population	Remarks
I	88	119	573	Average no. of inhabitants per building :—6·5. " " " " house :—4·3.
II	94	173	665	
III	83	137	642	
IV	123	164	628	
Total	388	593	2508	

A detailed study was made of the rat population of the village. This was done by placing rat traps in the buildings in rotation according to the census enumeration, about 230 traps being set each week. Table XVIII shows the number of rats caught alive and found dead each



86



Warli village.



Inhabitants of Warli.



TABLE XVIII.

Weekly summary of rats caught alive and found dead in Worli Village.

Date	Alive						Dead						Plague infected
	<i>rattus</i>	<i>decumanus</i>	<i>Nesokia</i>	Mice	Musk rats	Total	<i>rattus</i>	<i>decumanus</i>	<i>Nesokia</i>	Mice	Musk rats		
22nd to 26th Nov. '05	215	0	1	0	50	266	0	0	0	0	0	0	0
27th Nov. to 3rd Dec. '05	155	0	1	8	90	254	0	0	0	0	0	0	0
4th to 10th Dec. '05	128	0	0	0	33	161	0	0	0	0	0	0	0
11th to 17th "	159	0	0	0	53	212	0	0	0	0	0	0	0
18th to 24th "	103	0	0	0	44	147	0	0	0	0	0	0	0
25th to 31st "	96	0	0	0	43	139	4	0	0	0	0	0	0
1st to 7th Jan. '06	90	0	0	0	59	149	1	0	0	0	0	0	0
8th to 14th "	125	0	0	0	66	191	0	0	0	0	0	0	0
15th to 21st "	98	0	0	0	23	121	0	0	0	0	0	0	0
22nd to 28th "	74	0	0	0	0	74	0	0	0	0	0	0	0
29th Jan. to 4th Feb. '06	43	0	0	0	22	65	0	0	0	0	0	0	0
5th to 11th Feb. '06	57	0	0	0	16	73	0	0	0	0	0	0	0
12th to 18th "	41	0	0	0	32	73	0	0	0	0	0	0	0
19th to 25th "	45	0	0	0	4	49	0	0	0	0	0	0	0
26th Feb. to 4th March '06	41	0	0	0	2	43	2	0	0	0	0	0	0
5th to 11th March '06	29	0	0	0	3	32	0	0	0	0	0	0	0
12th to 18th "	39	0	0	0	0	39	0	0	0	0	0	0	0
19th to 25th "	24	0	0	0	0	24	0	0	0	0	0	0	0
26th March to 1st April '06	20	0	0	0	0	20	0	0	0	0	0	0	0
2nd to 8th April '06	33	0	0	0	0	33	1	0	0	0	0	0	1 <i>rattus</i>
9th to 15th "	20	0	0	0	0	20	0	0	0	0	0	0	0
16th to 22nd "	41	0	0	0	0	41	1	0	0	0	0	0	0
23rd to 29th "	49	0	0	0	0	49	0	0	0	0	0	0	0
30th April to 6th May '06	50	0	0	0	5	55	0	0	0	0	0	0	0
7th to 13th May '06	34	0	0	0	9	43	1	0	0	0	0	0	0
14th to 20th "	43	0	0	0	6	49	0	0	0	0	0	0	0
21st to 27th "	49	0	0	0	2	51	0	0	0	0	0	0	0
28th May to 3rd June '06	21	0	0	0	10	31	0	0	0	0	0	0	0
4th to 10th June '06	16	0	0	0	4	20	0	0	0	0	0	0	0
11th to 17th "	52	0	0	0	0	52	0	0	0	0	0	0	0
18th to 24th "	59	0	0	0	10	69	0	0	0	0	0	0	0
25th June to 1st July '06	30	0	0	0	2	32	0	0	0	0	0	0	0
2nd to 8th July '06	27	0	0	0	2	29	0	0	0	0	0	0	0
9th to 15th "	21	0	0	0	2	23	0	0	0	0	0	0	0
16th to 22nd "	18	0	0	0	7	25	0	0	0	0	0	0	0
23rd to 29th "	28	0	0	0	5	33	0	0	0	0	0	0	0
30th July to 5th Aug. '06	35	0	0	0	7	42	0	0	0	0	0	0	0
6th to 12th Aug. '06	18	0	0	0	7	25	2	0	0	0	0	0	0
13th to 19th "	27	0	0	0	5	32	4	0	0	0	0	0	0
20th to 26th "	14	0	0	0	0	14	0	0	0	0	0	0	0
27th Aug. to 2nd Sept. '06	22	0	0	0	0	22	0	0	0	0	0	0	0
3rd to 9th Sept. '06	43	0	0	0	2	45	0	0	0	0	0	0	0
10th to 16th "	46	0	0	0	8	54	0	0	0	0	0	0	0
17th to 23rd "	54	0	0	0	4	58	0	0	0	0	0	0	0
24th to 30th "	22	0	0	0	1	23	0	0	0	0	0	0	0
1st to 7th Oct. '06	49	0	0	0	0	49	0	0	0	0	0	0	0
8th to 14th "	33	0	0	0	0	33	0	0	0	0	0	0	0
15th to 21st "	9	0	0	0	0	9	0	0	0	0	0	0	0
22nd to 28th "	38	0	0	0	4	42	0	0	0	0	0	0	0
29th Oct. to 1st Nov. '06	25	0	0	0	0	25	0	0	0	0	0	0	0
Total	2608	0	2	8	642	3260	16	0	0	0	0	0	1 <i>rattus</i>

week; Table XIX gives the number of *M. rattus* trapped each fortnight, the number of traps set to capture these rats and, calculated from these figures, the number of rats caught for 100 traps set. The tables show that 2608 *M. rattus* were caught alive during about one year's operations. This number, it will be seen, is larger than the total human population of the village. In spite of the capture and destruction of so many rats, still a considerable number remained in the village at the end of the operations. If the rats caught may be regarded as

TABLE XIX.

Date	<i>rattus</i> caught	Traps set	No. of rats per 100 traps
22nd Nov. to 3rd Dec. '05	370	526	70
4th to 17th Dec. '05	287	590	49
18th to 31st „	199	577	35
1st to 14th Jan. '06	250	684	31
15th to 28th „	172	702	25
29th Jan. to 11th Feb. '06	100	447	22
12th to 25th Feb. '06	86	537	16
26th Feb. to 11th March '06	70	351	20
12th to 25th March '06	63	398	16
26th March to 8th April '06	53	267	20
9th to 22nd April '06	61	378	16
23rd April to 6th May '06	99	540	18
7th to 20th May '06	77	554	14
21st May to 3rd June '06	70	483	15
4th to 17th June '06	63	471	13
18th June to 1st July '06	89	472	19
2nd to 15th July '06	48	401	12
16th to 29th „	46	480	10
30th July to 12th Aug. '06	53	308	17
13th to 26th Aug. '06	41	308	13
27th Aug. to 9th Sept. '06	65	306	21
10th to 23rd Sept. '06	100	477	21
24th Sept. to 7th Oct. '06	71	443	16
8th to 21st Oct. '06	42	308	14
22nd Oct. to 1st Nov. '06	63	376	17
Total	2608		

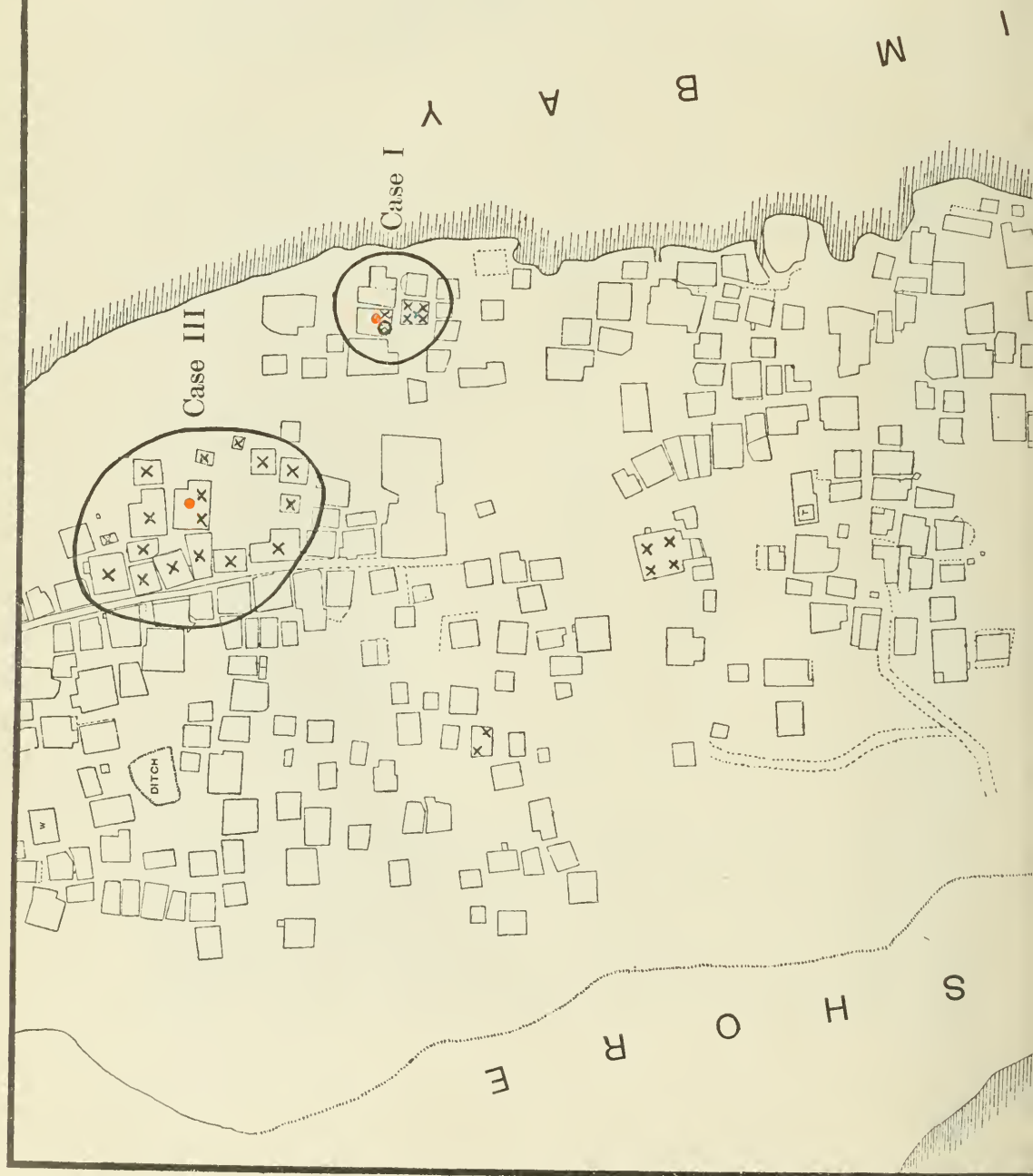
a fair index of the rat population it would appear that at the end of the year's operations the rat population of the village was reduced to about one quarter of the original population, that is to say, whereas in the first month of trapping 60 rats were caught per 100 traps set, in the last month only 15 rats were caught per 100 traps set. Table XIX may be compared with the similar table (X) made for Parel. In the case of this latter village the rat population after persistent trapping appeared to

MAP X

WARLI

Shows three cases of plague and events  
connected therewith







## WARLI

Shows three cases of plague and events connected therewith

Scale 100 feet to five-eighths of an inch

### Conventional signs used

- X Guinea-pig placed in house
- ⊗ Guinea-pig became plague infected
- Human plague case
- Plague infected rat



be reduced to about one half of the original population. The number of traps set in the latter half of the operations in Parel was, however, considerably curtailed and the total number of traps set was smaller than in Worli, the figures being 9391 and 11,390 respectively.

Five months after ceasing operations in Worli the villagers appealed to us to continue the routine setting of traps. They recognised that during the operations the rat population was considerably reduced, but after ceasing the work for five months the rodents had again become a perfect pest<sup>1</sup>. The fecundity of *M. rattus* is so great, that it is obvious that nothing but extensive and persistent efforts at their destruction can materially reduce their numbers in any village.

The complete absence of any sewage system in Worli is associated with the complete absence of *M. decumanus* from the village. Only two *Nesokia bengalensis* were captured. The number of mice caught was very small, probably owing to the fact that the rat traps used in this village permitted of their escape more readily than those used in Parel village.

The number of dead rats found and examined by us was only 16. Of this number only one was plague infected. Of the 2608 *rattus* captured alive, none were plague infected.

We are now in a position to consider the three events of importance in connection with plague to which we referred in the early part of this paper. These events we may detail under the names of the patients associated with them, viz.

I. The case of Jankibai.

II. The case of Bhagri.

III. The case of Maribai.

I. *The case of Jankibai.*

Jankibai was a woman close on 70 years of age who lived in Worli. She left the village in perfect health on the 14th February to attend the funeral of her nephew, Laxuman Narayan, who lived at 94-96 Sonapur Street in Bombay City. Motiram Ramji, another relative of the old woman, was ill with plague in the same house in which Laxuman had died. After the funeral Jankibai remained on at Sonapur Street nursing this latter patient. Dead rats had been found in the house at Sonapur Street, and many dead rats, found in the neighbourhood at this time, were proved by us to be plague infected. Motiram died on the 18th February. Jankibai in the meanwhile had developed fever. She

<sup>1</sup> This observation suggests that the rat population was really materially reduced by the trapping and not merely rendered more cautious.

came back to Worli on the 19th suffering from plague, as evidenced by high fever and a well-marked bubo in the groin. She went to a house in Block I, building No. 54, where we saw her on the 21st February. On visiting this house we observed that the building opposite, namely building No. 53, was vacant. On inquiring we learned that the inhabitants had found a dead rat in this house on the 11th February, and had in consequence vacated and were living in an adjacent temporary shed.

Two days after Jankibai came to the village, we placed two guinea-pigs in her house and four guinea-pigs in the vacated house opposite. The guinea-pigs were kept in the houses from 21st to 24th February, being examined for fleas on four occasions. The two guinea-pigs in Jankibai's house gave eleven rat and four cat fleas. The four guinea-pigs in the house in which the rat had been found gave two rat fleas, eleven cat fleas and one *Sarcopsylla*. One of the two guinea-pigs which had been placed in Jankibai's house after isolation in the laboratory died of plague on the 9th March. All the other guinea-pigs remained healthy.

Jankibai's house evidently harboured infection, whereas in the house opposite, from which the inhabitants had fled owing to the finding of a dead rat, we failed to discover infection. The cause of death of this rat could not be ascertained. The inhabitants of Worli are so strongly impressed, after bitter experience, with the relation that exists between the finding of a dead rat and the later development of human plague cases, that during the plague season from January to May the finding of a dead rat causes alarm. The inmates of the building, in which the rat has been found, generally vacate it and live in temporary quarters adjacent to their home. The finding of a dead rat at any other season would be regarded with indifference. In addition to the above instance, on three other occasions we found that the inhabitants had vacated their homes because a dead rat had been found. Although the rats on these four occasions were not examined by us, having been disposed of or removed by a kite or cat, we placed guinea-pigs in the vacated buildings. In no instance, however, did any of these guinea-pigs become infected with plague. The four buildings are shown on Map X, the rats being indicated by green dots and the guinea-pigs, which were placed in the houses, by black crosses. On several other occasions before the events we are now considering, rats which had been found dead were examined by us, but were found not to be infected with plague. Moreover, the examination of 1343 rats caught alive up to this time, did not reveal the existence of plague among the rats of the village.



Taking all the facts into consideration it seems to us that the guinea-pig which contracted plague in Jankibai's house probably derived its infection from infected fleas imported by Jankibai from Sonapur Street in Bombay City. This case closely resembles one recorded in the account of our observations in Parel village. In this instance, it will be remembered, another such woman, coming from the city, appeared to have brought infected fleas to that village (Case 1; Parel village).

For some time after this no event of importance in connection with plague occurred in Worli. About the end of March plague broke out in the fishing village of Sewri, situated on the eastern side of the island. Some of the inhabitants of this village migrated to Worli where they had relations and friends living. We placed guinea-pigs in two of the houses in Worli, in which the Sewri people were residing. These guinea-pigs, however, remained healthy.

## II. *The case of Bhagri.*

On the 29th of March we received information of a second case of plague in Worli village. The patient was a schoolboy, named John Domenick Bhagri. He attended a school in the Dadar district of Bombay. As the parents of the lad assured us that no rats had been found dead in their house, we visited the school and obtained the following history from the headmaster. A dead rat had been found in a class room of the school on the 23rd March. Bhagri, however, had not been in this room. On the morning of the 26th March, when the boys were playing on the playground, one of them saw a dead rat lying on the ground. He shouted to Bhagri "Kick it out"; which instruction Bhagri immediately proceeded to carry out. On the following day Bhagri went to school as usual, but in the afternoon on account of feeling unwell had to ask leave to go home.

Bhagri's condition precluded our obtaining confirmatory evidence of this story from himself, but some of the schoolboys we saw vouched for its truth. On our visiting Bhagri at his home on the 2nd April we noticed that the next house had been vacated, and on inquiry ascertained that a dead rat had been found in it on the 20th March. On the same morning, namely 2nd April, we found a dead rat lying on the road close to Bhagri's house. This rat on examination was proved to be plague infected.

Two hypotheses may be brought forward in connection with the question of where Bhagri became infected:—(1) it is possible that he acquired the disease at the school on or about the 26th March and

carried with him to his home infected fleas, which conveyed the disease to the rat we found on 2nd April; or (2) it is possible that he derived his infection in the village from an epizootic of plague, which was evidenced in the dead rat found on the 20th March and again in the rat proved to be plague infected and found by us on the 2nd April.

With the object, if possible, of getting further evidence on this question we carried out some guinea-pig experiments.

On the 2nd April 25 guinea-pigs were placed in 21 houses surrounding Bhagri's house, while at the same time two guinea-pigs were placed in Bhagri's own house. The guinea-pigs were left in the houses for a fortnight. They were then returned to the laboratory. All of them remained healthy. It is probable, therefore, that no epizootic existed amongst the rats in this neighbourhood, as our experience in Sion village had shown us that experiments done with guinea-pigs placed in houses in this manner are a very delicate test of the existence or not of plague amongst the rats. It, therefore, appears likely that Bhagri obtained his infection at the school, possibly when he kicked the dead rat, and that the rat found by us acquired its infection from fleas imported to the village by Bhagri. A very similar experience has been recorded by us in connection with Wadhala village. In this case it appeared probable that coolies, employed by the Commission and who frequently came in contact with infected rats, conveyed the disease to a single rat which was found dead close to their house.

Bhagri died on the 5th April. No other case of plague occurred in this part of the village.

### III. *The case of Maribai Anton.*

We have now to record the last case of plague which occurred in Worli. Maribai Anton lived in Block I, building 70. She had always lived in the village and was not in the habit of leaving it, except for a day once a fortnight, when she visited Mahim or Sewri to sell fish. She returned from one of these visits to Sewri feeling ill on the 28th March. She developed a femoral bubo and died of plague on the 3rd April. No rats had been found dead in her house or in any of the adjoining houses. On the 4th April two guinea-pigs were placed in Maribai's house and one in each of fifteen houses in the neighbourhood (*vide* Map X). The guinea-pigs were allowed to remain in these buildings for 10 days and were thereafter isolated in the laboratory. They all remained healthy. It is probable, therefore, that Maribai acquired her infection outside the village. We know that she had visited Sewri, which was at this time badly infected; but if she became infected there, the incubation period

of the disease must have been less than 12 hours. Such a short incubation period in connection with plague has been noticed by others.

*Summary and Conclusions.*

(1) Worli village is badly infested with rats, practically all *M. rattus*.

(2) Persistent trapping for one year appeared to reduce the rat population to approximately one quarter of the original number.

(3) Three cases of plague which occurred in the village were investigated. It is probable that these three cases all contracted the infection outside the village.

(4) In two instances there is evidence which points to infection having been introduced in the clothing or persons of people, and of this spreading in one instance to a guinea-pig, and in the other instance to rats.

## XXIV. GENERAL CONSIDERATIONS REGARDING THE SPREAD OF INFECTION, INFECTIVITY OF HOUSES, ETC. IN BOMBAY CITY AND ISLAND.

- I. Introduction.
- II. The spread of the infection within houses.
  - (1) The question of the spread of infection by direct contact with a suffering case.
  - (2) The question of the infectivity of houses.
- III. The transportation of infection to a distance.
  - (1) Transportation of infection in clothing and merchandise.
  - (2) Importation of infection into a hitherto uninfected locality.
- IV. The question of the occurrence of plague in domestic and other animals.
- V. Summary and conclusions.

### I. INTRODUCTION.

At this point the question arises: Do modes of spread, other than by means of the epizootics, exist which aid in the diffusion of the infection during epidemic prevalence of the disease—in other words, is the epidemic wholly or only partially dependent upon the epizootics?

In the following pages we shall attempt to supply an answer to this question. Before entering into details we may for the sake of clearness indicate the principal points about to be discussed.

In the first place, it is necessary to examine the statement, that the epidemic is to an appreciable extent due to infection acquired by direct contact with patients suffering from the disease. This statement involves the assumption, that the plague patient is not infrequently a source of danger to others on account of the infectivity of his excreta. Closely connected with this is the question of the infectivity of houses and the nature of the infecting agent within them. It is conceivable, and indeed it has been suggested, that the infectivity of houses is referable to contamination of the soil (*e.g.* cowdung floors) with *B. pestis* derived from the excreta of plague-infected rats or men. Again, it is conceivable that the infection resides for a time in articles, *e.g.* bedding and clothing, which have been soiled by the excreta of patients suffering from plague.

Lastly, there is the view that the infection in houses is present in an effective form in the bodies of rat fleas.

It will be noted that these problems have reference to the spread of the infection within houses. Not less important, however, is the problem of the transportation of infection to a distance in clothing or in merchandise. The question of the importation of infection into a hitherto uninfected locality altogether hinges upon the possibility of such a mode of spread.

In conclusion, we shall give the results of our experience in the matter of the occurrence of plague in domestic and other animals (excepting rat plague) and shall discuss, briefly, the significance of animal plague on the spread of the epidemic.

## II. THE SPREAD OF THE INFECTION WITHIN HOUSES.

### (1) *The question of the spread of infection by direct contact with a suffering case.*

In this connection it is our intention to consider the alleged spread of infection from patients suffering from the bubonic and septicaemic varieties of plague. The contagiousness of pneumonic plague<sup>1</sup> and its mode of transmission have never been disputed, but on account of its rarity, this type of the disease plays only a minor part in the spread of the epidemic. Our evidence with regard to the question at issue is derived from various sources and may be arranged as follows:

#### (a) *Experience in hospitals.*

When plague broke out in Bombay ten years ago it was not long before it was recognised that the attendants in plague hospitals remained singularly free from danger of infection, although they were brought frequently into intimate contact with patients in the acute stage of the disease. This is the more remarkable, when it is borne in mind that in the majority of fatal cases there is incontinence of urine and faeces, and that the hospital patients and the menial staff who attend them do not possess an elementary notion of the most ordinary precautions.

Our own experience amply bears out the view that the wards of a plague hospital are devoid of infectivity. Our visits to the Maratha

<sup>1</sup> We are aware of the possibility that cases of primary pneumonic plague may originate from septicaemic cases with secondary pneumonia, but we have no observations on the point.



plague hospital in the course of certain investigations connected with our work strongly impressed on us the truth of the dictum, that one of the safest places during the epidemic is the ward—the “acute ward” we might add—of a plague hospital. In addition certain experimental observations we have made go far, in our opinion, to prove that a plague hospital ward is devoid of infectivity and that the excreta of patients suffering from plague are not, as a matter of fact, infective from the point of view of epidemic plague.

Three modes of experimentation were adopted.

First, two guinea-pigs were introduced into the “acute” ward of the hospital in the epidemic season and were allowed to run freely about the ward. The guinea-pig, as is abundantly evident from the numerous experiments already related, is markedly susceptible to the natural infection of plague, and yet these animals, although kept in the ward for one week, remained perfectly healthy. No rat fleas were caught on them. The second experiment constituted a still more severe test. 15 guinea-pigs were confined in a flea proof godown in the laboratory compound, and bedding, recently soiled by the excreta of acute cases just before death, was added daily, each lot of bedding being kept in the godown for 24 hours. Although the experiment was continued for several weeks, and in spite of the intimate contact with this material in a confined space, none of the guinea-pigs contracted the disease. The third method was to rub the urine or faeces of acute plague cases into a scarified area on the abdomen of guinea-pigs. A considerable number of experiments were carried out but only one doubtful success (by rubbing in faeces) was obtained.

(b) *The influence of imported cases on the spread of the epidemic.*

By an imported case we mean a patient who has acquired infection in a place, *e.g.* at his work, other than the house in which he is found suffering. We would also wish it to be understood in this connection, that there is no evidence of rat mortality due to plague in the house in which the patient is found. If surveillance were kept on the relatives and attendants of such a case, it should be possible to discover whether any plague cases followed amongst the contacts. It must be admitted that the careless habits of the people, to which allusion has been made, offer abundant opportunities for direct transference of infection by contact, were such a method of infection an effective one. We have no evidence, however, that this method of infection, which we can merely conceive of as occurring, is ever effective in Bombay, and we

shall now proceed to support this conclusion by relating our experience of a number of imported cases in the outlying villages. The cases were personally investigated by members of the Commission.

In Parel village 28 cases of plague occurred in the epidemic under review. These cases are especially appropriate to our present purpose, since the epizootic in the village was a very limited one, and since the available evidence shows, that of the total cases 17 fall into the group of imported cases. In reviewing the entire series of 28 cases, it is noteworthy that only in four instances did two or more cases occur in the same house. In all the houses in this village in which plague cases occurred a number of individuals were living in the same room as the sick, but in no instance did we obtain any evidence to show that the sick communicated the disease to their attendants and friends.

In Wadhala village we inquired into two cases of plague which were imported from the City. One of the patients was brought to the village during his illness, while the other was attacked with the disease on the day after arrival. There was no evidence of rat mortality in either of the buildings in which the cases were found. Guinea-pigs placed in each of the houses remained healthy, and no cases occurred amongst the persons who were living in either of the houses.

Worli village furnished similar examples (*vide* detailed description of Worli).

(c) *Occurrence of single and multiple cases in houses and buildings.*

If plague is an infectious disease, it would certainly happen that multiple cases in a house would be common. It is obvious, then, that an investigation of the relative frequency of single and multiple cases in houses might throw light on the question of the spread of infection by contact with a suffering case.

Our own experience has been, that it is comparatively rare to find two or more cases in a house. Our attention has been specially directed to the point, because throughout the epidemics of 1906 and 1907 we made a continual endeavour to find instances of this kind with the purpose of using such houses for guinea-pig experiments.

In addition we have analysed a large mass of data relating to this question drawn from the records of the epidemics 1903—1906, inclusive. Before calling attention to the results of this analysis, we may explain that the data were abstracted from records kept by the District Registrars. Each District Registrar has a street register, which contains a list of the street numbers of every inhabited *building* in the sections

under his charge. When he is informed of a case of plague in a building (as well as of certain other diseases), a note is made of the fact in the register opposite the corresponding building number, the date of notification being also recorded. No data are, however, available in these street registers for showing the number of cases which occur in a *house* in any of the buildings. From these records we have abstracted and analysed the data relating to plague for ten of the sections in the City for four successive years.

We may now draw attention to the figures of principal interest in the table which gives the results for the year 1906 (*vide* Table I). The columns showing the average number of inhabitants per building and the average number of houses per inhabited building are important, since they give an indication of the average size of the buildings in each section. It will be observed that the yearly records have been subdivided for our purpose into two half yearly periods, January to June, and July to December. This division practically corresponds to the plague season and the off-plague season in Bombay. Attention is directed to the column which shows the average number of cases per building for each section. The columns in the table towards the right hand are important since they give the percentages of buildings in which single and multiple cases occurred in each half year.

The results which come out of a study of the tables as a whole are striking. It will be noted, in the first place, that the average number of cases per building in the epidemic months for all the sections given in the table is very low, never indeed rising to three per building.

This low average of cases per building, when considered in the light of the fact that the buildings on the whole have a large population distributed as separate families in houses within the buildings, is without doubt good evidence that the average number of cases *per house* must be a very low figure. It is only necessary to add that the columns showing the percentages of buildings which yielded single and multiple cases confirm this view.

Taken as a whole the evidence afforded by the tables seems to us to accord completely with that already adduced.

Since it cannot be questioned that multiple cases in families do occasionally occur, it becomes necessary to furnish an explanation of the source of infection of such cases.

It will readily be understood that, even if no spread of infection by contact occurs, one might still expect to meet with multiple cases if the plague rat and the rat flea be regarded as the common source of



TABLE II.

Serial No.	Address	Dates of attack of cases	History of dead rats	Remarks
1	60, Bellasis Road	4 plague cases between 8/3/07 and 12/3/07	2 dead rats about 5/3/07	On 16/3/07, 7 rat fleas got on 1 guinea-pig.
2	159, Queen's Road	6 plague cases between 12/2/07 and 19/2/07	Many dead rats before cases	On 20/2/07, 11 rat fleas got: 3 of these contained plague bacilli in stomach contents.
3	11-13, Kombhal Lane	3 plague cases all on 25/3/06	Dead rat in room. Date ?	White rat in cage unprotected from fleas died of plague.
4	85, Bunganga	(a) 3 plague cases all on 5/4/06 (b) 2 " " both on 5/4/06	(a) None (b) Dead rats on 4/4 and 10/4/06	(a) Nil. (b) 25 rat fleas got on guinea-pig; this guinea-pig died of plague.
5	4, Kalachawki Rd	4 plague cases—2 on 13/4/06 2 on 14/4/06	Dead rats found; date ?	72 rat fleas got on 2 guinea-pigs both of which died of plague.
6	22, Navroji Hill North	(a) 3 plague cases—2 on 14/4/06 1 on 15/4/06 (b) 3 " " 14/4, 15/4, 16/4/06	(a) Dead rats in adjoining room; date ? (b) Dead rats in adjoining room	(a) 8 rat fleas got on 2 guinea-pigs. (b) 47 rat fleas got on 2 guinea-pigs.
7	1, Ripon Road	4 plague cases, 15/4, 2 on 16/4, 1 on 17/4/06	Dead rat; date ?	170 rat fleas got on 2 guinea-pigs, one of which died of plague.
8	Jubilee Mills Sevri (cotton godown)	5 plague cases all about 17/3/07	Several dead rats before cases	On 25/3/07, 6 rat fleas got on 2 guinea-pigs, one of which died of plague.
9	96, Cavet (infection in kitchen)	Cooks { 1st case, 17/3/06, left day of attack 2nd case, 19/3/06, removed to hospital Cook's assistant, 3rd case, 12/3/06, removed same day Child in house, 4th case, 20/3/06 Assistant in kitchen, 5th case, 21/3/06	None	106 fleas got on 2 guinea-pigs, one of which died of plague.



infection. We think that such cases are to be explained in this way. In confirmation of this view it may be noted, that in our experience the evidence regarding rat mortality was much stronger in multiple cases than in single cases in a room. Another important point is, that as a general rule the persons in the multiple cases were attacked almost simultaneously, as if by a common infecting agent. In order to illustrate these points we have brought together in Table II the essential details of a series of multiple cases which occurred in a number of badly infected houses. From a study of these cases, in the light of our knowledge of the incubation period of the disease, it is evident, that all are aptly explained on the view of a common source of infection: indeed in some this is the only possible explanation. Moreover, in nearly every instance a history of dead rats was obtained and in several instances the houses were proved to be infective, the infectivity being associated with the presence of rat fleas in unusual numbers within them.

- (d) *The question of the transmission of infection from a septicaemic human case to the attendants of such a case by the agency of the human flea.*

Experimenting with human fleas (*P. irritans*) we obtained three successful transmissions out of 38 experiments, *the fleas having previously been fed on selected septicaemic rats* (vol. VII. p. 413). We have also shown that multiplication of plague bacilli takes place in the stomach of the human flea. Taking into consideration the evidence relating to the spread of infection by direct contact adduced above, and further taking into account the slight septicaemia as observed microscopically and by cultural methods in human cases compared with that in rats (vol. VI. pp. 521, 527), we think that transmission of infection from man to man by means of the human flea is probably a very infrequent occurrence.

We may add that there is in our view still less reason to believe that infection is transmitted by infected human fleas to rats in houses, because we know that this species of flea is very particular in its choice of host, that it does not live well upon the rat, and that it will only attack this animal in the absence of its proper host.

- (e) *The question as to whether an epizootic amongst rats in houses is alone sufficient to account for a widespread dissemination of infection throughout a locality.*

It is our experience that a widespread dissemination of infection in houses may result from an epizootic in the rats, even when conveyance of infection by direct contact with sick occupants of the houses is rigidly excluded. This conclusion is based upon a study of plague infection in an evacuated village, namely, Sion Koliwada (*vide* report on this village). In this village an experiment was carried out, which showed that a large proportion (at least 45%) of the total buildings became infective in consequence of an epizootic amongst the house rats. The possibility that infection by direct contact with sick animals played a part in the spread of the infection was definitely excluded by their isolation in the houses.

- (f) *Conclusion derived from a consideration of the evidence which has been brought forward on the question under discussion.*

A review of the whole of the evidence bearing upon the question at issue leads us to conclude, that contact with plague cases, although a conceivable mode of spread of infection, yet, as a matter of fact, plays no part in the spread of the epidemic.

(2) *The question of the infectivity of houses.*

A. *General considerations.*

Most investigators are agreed that the infection of plague is characteristically present in buildings, in other words, that plague is a place infection. Thus, it has been an oft-repeated observation, especially in India, that healthy persons, who have not otherwise been exposed to infection, have contracted plague after visiting houses vacated because of the disease. Again, it is well known that the evacuation of an infected village by its inhabitants and their removal to a temporary camp, if only a short distance away, is one of the best measures for checking an outbreak of plague. A case of this kind happened in Sion Koliwada village. When plague broke out in this village, almost all the inhabitants voluntarily vacated their houses and went to live in a camp of rude huts only about 200 yards distant. One or two cases of plague occurred after the people were in camp, but these we had reason to attribute to infection received during a visit to the vacated houses for domestic purposes.

B. *The infectivity of houses.*

(a) *The nature of the infecting agent in houses.*

The conclusion that infection by direct contact with a suffering case plays no part in the epidemic spread of the disease, that is, that the excreta of a plague patient have little or no infective properties, is a very important one, because it simplifies the problem of the infectivity of houses and the nature of the infecting agent within them. It is evident that, having come to this conclusion, no importance can be attached to contamination of the soil by such excreta. For, if excreta in a fresh state on clothing and bedding possess no infective properties, it would appear very improbable, that the transference of bacilli from this source after being deposited on, *e.g.* a cowdung floor, would prove effective. From similar considerations, and taking into account the relatively small bulk of rat excreta compared with human excreta, we conclude, further, that contamination of any part of a house or its furnishings by the excreta of rats plays no part in the spread of the epidemic. Apart from reasoning of this kind certain experiments carried out by us in infected houses strongly support the view that the infectivity of houses cannot be referred to soil, contaminated by infection in the form of excreta, either of men or of rats, deposited casually on any part of floors. We refer to experiments, in which susceptible animals—guinea-pigs, white rats, monkeys—were confined in cages of special construction in which the animals were protected from possible contamination of the soil. Moreover certain experiments in which guinea-pigs were confined in wire cages appeared to us to be more frequently successful, than they could possibly have been if the infection, assuming it to be effective, had been thus casually deposited.

The arguments adduced above force us, thus, to seek for the source of the infectivity of houses in some intermediate agent, which is capable of conveying the infection from rats to man. Our view is that this intermediary is the rat flea and that the infectivity of houses is due to the presence within them of infected rat fleas. It is unnecessary in this place to enter fully into the evidence for this conclusion, but we may refer the reader to certain papers which have already been published dealing with experiments in plague houses.

(b) *Circumstances which may be accepted as evidence of infectivity in houses.*

Definite proof of infectivity is forthcoming, when one or more of the following results are obtained :

(1) The discovery of one or more dead plague-infected rats in any part of a house.

(2) The death from plague of a guinea-pig allowed to run free in a house.

(3) The capture of plague-infected fleas in a house, that is to say, the demonstration of abundant bacilli microscopically indistinguishable from *B. pestis* in the stomach contents of fleas caught on guinea-pigs which are allowed to run free in a house.

(4) The death from plague of a previously healthy guinea-pig in a flea proof cage, to which animal fleas caught on a dead rat or on guinea-pigs allowed to run free in a house had been transferred.

Presumptive evidence of infectivity rests upon (1) the occurrence of one or several plague cases in a house, and (2) a definite history of mortality amongst rats in the house, especially during the plague season. The evidence is strengthened when multiple cases occur in a house associated with a history of rat mortality. We may add that the capture of a large number of rat fleas, in our experience roughly from 20 to 200, on guinea-pigs placed in suspected houses affords a presumption of infectivity. It must be kept in mind, however, that the number of fleas caught in houses proved to contain infection depends largely upon whether the guinea-pig is put into the house shortly after the death of the rats or at a later period. The largest numbers are obtained in the former case.

Again, even if there is no history of dead rats in a house, the discovery of plague rats or even a history of dead rats in the building, in which the house is situated, must be held to be matter of evidence in an inquiry into the infectivity of houses in buildings. Although necessarily of less value as evidence the occurrence of plague rats in the adjoining gully or in the vicinity of a building must also be considered as carrying weight in a similar inquiry. It must be noted that in this statement we are referring to the conditions which obtain in Bombay.

(c) *Certain features of the infectivity of houses.*

It is noteworthy that the infection in houses is frequently localised to a part, sometimes even a very small part, of a house and that the



infection, as one might expect, varies in "concentration" in different houses.

As to the first point, it has been our experience that the infection may be confined to a single room in a house consisting of several rooms. We may cite the case of a severe outbreak (five cases) in a family living in 96 Cavel (see Table II). In this house the infection was confined to the kitchen. No fewer than 106 fleas were got on two guinea-pigs placed in this room, one of the animals subsequently dying of plague.

In a paper on the life history and habits of fleas it will be pointed out that plague sick rats frequently harbour fleas in unusual numbers, and that such rats in their wanderings are apt to leave a trail of infected fleas behind them. Fleas, if dropped under these circumstances in the living room of a house, might easily prove a source of danger to man. The danger is, however, much greater in the immediate neighbourhood of a rat dead of plague, because here the infection is in a concentrated form on account of the larger number of fleas which remain at or near the spot.

The statement that the degree of infectivity of houses is proportional to the number of rats which die of plague in them would seem to require no proof.

(d) *Duration of infectivity of houses.*

The duration of infectivity of houses is probably very variable, depending as it does on the persistence of the infection amongst the rats. In one instance which came under our notice the interval between the discovery of the first and the last plague rat was as long as 13 days.

It has also to be kept in mind that houses are liable to reinfection by rats when the epizootic is very widespread, as in Bombay.

It has been alleged that the infection may persist in a house or in a locality apart from rats. The underlying idea in this belief appears to be that the *B. pestis* is able to live for long periods, in soil for example, and that the infection may continue in a latent form in this medium, until the next plague season when it breaks out afresh in a virulent form. Associated with this highly speculative assumption is the idea that the conditions in certain houses are especially favourable for the persistence of infection, so that these houses are attacked with plague year after year. It seems to us that statements of this kind are entirely without value, unless supported by systematic and long continued observations of the course of the infection amongst the rats. Further, the whole of our experience in Bombay is opposed to the view that the



infection persists for any length of time in a house or locality apart from infection amongst the rats. A number of houses which were plague infected during the epidemic of 1906, were experimentally investigated at regular intervals during a period of one year but no evidence of persistence of infection was forthcoming in any of them, nor did plague occur in them during the subsequent epidemic season.

### *C. The infectivity of buildings.*

In Table III figures are set forth which show the incidence of plague on persons living on the various floors of buildings for the whole of Bombay. It will be noted that the population living on the various floors is expressed as percentages of the total population and that similarly the plague cases investigated by us have been grouped according to their occurrence on different floors, the resulting numbers being also expressed as percentages of the total number of plague cases. Comparison of the percentage figures for each floor makes it evident that they correspond in a remarkable manner. Similar figures have been calculated for 21 of the sections and generally speaking they confirm the accuracy of the results for the whole of Bombay. We think it, therefore, justifiable to make the general statement that the incidence of plague on persons living on different floors of buildings is the same.

Further, it has been our experience that when infection is present in several houses in a building the incidence of plague on these houses appears to have followed no definite course. This irregularity is readily explicable from the point of view of the epizootic. Apart from infection amongst the rats it would seem impossible to explain why one house in a building should be infected rather than another since the conditions within the houses are, as a rule, identical.

## III. THE TRANSPORTATION OF INFECTION TO A DISTANCE.

### *(1) Transportation of infection in clothing or merchandise.*

If we can exclude modes of spread of the infection of plague other than the rat flea, we must conclude that the transportation of infection to a distance is attributable solely to the conveyance of the infection in the rat flea. A little reflection suffices to show that transportation of infection in this medium is not only conceivable, but that under certain circumstances it may be a very likely contingency.

TABLE III.

*Incidence of plague on persons living on ground floor, 1st floor, etc.*

	Ground floor		1st floor		2nd floor		3rd floor		4th floor		5th floor		6th floor		7th floor		Total
	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	
Bombay																	
Population	351,429	48·9	189,066	26·3	107,827	15·0	50,786	7·0	16,382	2·3	20,58	0·4	184	0·02	18	0·003	718,650
Plague cases	4,879	49·2	2,637	26·6	1,369	13·8	714	7·2	183	1·8	28	0·3	5	0·05	83	0·8	9,898

TABLE IV.

*Incidence of plague on buildings classified according to number of storeys.*

	Buildings with ground floor only		Buildings with ground floor and 1 storey		Buildings with ground floor and 2 storeys		Buildings with ground floor and 3 storeys		Buildings with ground floor and 4 storeys		Buildings with ground floor and 5 storeys		Buildings with ground floor and 6 storeys		Buildings with ground floor and 7 storeys		Numbers on which calculated
	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	Number	P.c. on total	
Bombay																	
Total Buildings	20,608	53·6	7,147	18·4	5,149	13·3	3,341	8·6	1,904	4·9	618	1·6	74	0·2	2	0·05	38,843
Buildings plague infected	1,212	19·0	1,174	18·4	1,416	22·2	1,452	22·7	796	12·5	276	4·3	54	0·8	—	—	6,380

*(a) Transportation in merchandise, grain, etc.*

In an account of the bionomics of the rat flea to be published later various modes of dispersal of fleas are indicated. Two of these have a bearing upon the subject under discussion. They are: (1) dispersal of fleas with the host, when the latter is carried in merchandise, and (2) dispersal by means of merchandise, grain, clothing, etc., the host, however, not being transferred with the fleas.

With regard to the first point, we may note that we have seen rats dive, as it were, into bags containing grain, so that the bags could be moved without any evidence of the presence of rats within them.

It is further obvious that merchandise and grain, which have been visited by rats, may have rat fleas (possibly infected fleas) deposited in them, so that these fleas might be transferred to distant places. Examination on one occasion of bran, which was kept in a bin with a loosely fitting lid in a rat infested room, revealed the presence of numerous rat fleas in the bran. In this connection it must be noted that adult fleas in the absence of any host to feed upon rapidly die, generally in five days.

*(b) Transportation of rat fleas in clothing.*

Infected fleas may be transported in this way: (a) in the clothes of a person who has been for a time in a plague-infected house, and (b) in bundles of clothing or bedding removed from an infected house.

(a) A reference to a previous paper ("A note on man as a host of *P. cheopis*," vol. VII. p. 472) shows how readily and in what large numbers rat fleas may under certain circumstances come on to man. The experiments cited in this paper indicate that rat fleas may often be transported in this way from place to place, especially from plague-infected houses, where they are more likely to take to man because of the absence of their true host. During our visits to infected houses in Bombay City we had many opportunities of noting that we carried away fleas on our persons and on our clothing. These fleas were generally human fleas, but occasionally they proved to be *P. cheopis*.

(b) The following experiments which were carried out at the end of the epidemic of 1906 are of interest:

Bundles of clothing, bedding, etc. were sent to the laboratory from houses in the City in which plague cases had occurred. The bundles after being opened out were kept in a flea proof godown for several days, being replaced by fresh ones as they arrived. Along with the

clothing guinea-pigs were placed in the godown. In some instances the animals were allowed to run free, in other cases they were placed in pairs in cages, the control animal being protected from fleas either by means of a layer of tanglefoot or by a curtain of wire gauze.

In all 26 free guinea-pigs were exposed in the godown for an average period of about four days each. The result of the experiment was that three fleas (two rat fleas and one human flea) were caught on the animals and that one of the free guinea-pigs died of plague, the bubo being in the neck. On the tanglefoot of one of the cages three fleas were caught. The stomach contents of one of these fleas, a human one, contained abundant bacilli indistinguishable from *B. pestis*.

It would appear that the guinea-pig which died of plague was infected by fleas, because we proved in a similar experiment that clothing and bedding recently soiled with the excreta of plague cases possessed no infective properties. The experiment therefore shows:

(1) that rat fleas may be transported to a distance in bedding and clothing removed from plague houses; and

(2) that such rat fleas may prove infective if transferred to a susceptible animal in the place to which they are carried.

It ought to be added that these experiments were carried out at an unfavourable time, namely, towards the end of the epidemic, so that they give no indication of the frequency with which rat fleas may be transported in clothing, during the period of the epidemic when these insects are especially numerous.

(2) *Importation of infection into a hitherto uninfected locality.*

From the discussion of the transportation of infection to a distance we are led naturally to consider the question of the importation of infection into a hitherto uninfected locality.

In the first place, we would point out that in whatever way rat fleas are transported, whether in clothing or merchandise, they will select, when carried to their new surroundings, either their true host, *i.e.* the rat, or the next best available animal. If then infected fleas are imported into a house they will by preference attack the rat rather than the human occupants of the house. It is apparent from the account we have given of the rat infestation of houses in Bombay, that under such circumstances opportunities for transference of infected rat fleas to rats in houses are abundant. It would appear, then, that the introduction of infected rat fleas into a hitherto uninfected locality may lead to

serious consequences by giving rise to an epizootic amongst the rats. We may note, (1) that many chances render uncertain the effective transference of infection to rats by importation, (2) that the most favourable time for such infection to act effectively is when the conditions for epizootic prevalence are most favourable, namely, during the period of the rise of the epizootic, and (3) that in Bombay infection carried in this manner probably affects *M. rattus* more often than *M. decumanus*, since *M. rattus* is the species most intimately associated with man. As an example of the uncertainty of importation by infected fleas we may cite several cases which were investigated in Parel village. Seven rooms, in which nine imported cases were found, were tested by means of guinea-pigs. In one of these rooms a guinea-pig died of plague. From the available evidence we concluded that this guinea-pig was infected by rat fleas imported to the village from Bombay by the patient.

In Bombay excellent opportunities are afforded of observing the importation of infection by human agency in the case of the outlying villages, Sion, Wadhala, and Worli, which were specially investigated by us. These villages were indeed selected for study for the reasons that they occupied isolated positions and that their inhabitants followed an employment (as fishermen or agriculturists) which kept them for the most part confined to their villages. For these reasons it was considered that it might prove a comparatively easy matter to narrow down the inquiry into the origin of the epizootic and epidemic and to trace the infection, if imported, to a portion of Bombay City which was at the time infected. In our view the outbreaks of plague in these villages are due to a chance importation of infection from the City. Our reasons for so thinking are as follows:

First a systematic and extensive examination of the rats in the villages failed to reveal plague either in an acute<sup>1</sup> or in a chronic form, amongst these animals during the off-plague season.

Secondly, our own observations during two years and a study of the history of the outbreaks in previous years clearly show that the incidence of plague in these villages is extremely erratic, both in regard to time and place, as if due to a chance importation. Thus, one part or indeed the whole of a village may be badly infected one year but may escape altogether in the following year.

A complete account of the observations made in the villages is

<sup>1</sup> See p. 842.



given elsewhere. We may refer to four cases of imported infection, which illustrate the point under discussion, namely :

(a) *The origin of the epizootic in Sion Koliwada village in the plague season of 1906.*

(b) *The first plague-infected rat in Wadhala village.*

(c) *The case of Jankibai in Worli village.*

(d) *Case I in Parel village.*

In conclusion, we would point out that the carrier of the infection may not contract the disease, as the Sion and Wadhala cases show. It is interesting to compare this fact with instances, in which a guinea-pig allowed to run free in a house escaped infection, although the fleas taken on it, when transferred to a guinea-pig in the laboratory, killed the latter with plague.

#### IV. THE QUESTION OF THE OCCURRENCE OF PLAGUE IN DOMESTIC AND OTHER ANIMALS (EXCEPT RATS).

We have already noted that when rat fleas are starved they will readily attack any animal which is available to feed upon. From this consideration and from our observations in Bombay it would appear that the occurrence of plague in animals other than rats is to be explained solely by transference of infection from the rat to these animals by means of the rat flea.

We are of opinion that animal plague is of little or of no importance, if only for the reason that instances of this kind occur very seldom, at least in Bombay.

We have observed natural plague in guinea-pigs, rabbits and monkeys.

Liston described an epizootic of plague amongst guinea-pigs in Bombay which occurred in 1903, and again in 1905. These epizootics were associated with a history of dead rats, and rat fleas were found on the guinea-pigs. In 1906, an epizootic of plague broke out amongst a stock of guinea-pigs and rabbits in the laboratory. Plague-infected rats were found by us in the runs, and rat fleas were taken on the animals.

Only one suspicious case of plague in a cat has come to our notice. This animal had a purulent bubo in the neck, but no growth of *B. pestis* was obtained from the pus.

*Epidemic amongst the monkeys in Victoria Gardens.*

This epidemic occurred in the zoological and botanical gardens in the City.

The monkey house is built of stone with a high masonry plinth. The floor throughout is of patent stone and is quite impermeable to rats. There is a central passage with four cages on each side, in which the monkeys are confined. The cages are separated from one another by solid walls and are closed in in front by iron bars. For purposes of description they are numbered in the diagram, I—VIII.

The history of the epidemic is as follows:

On 11/4/06 two dead rats, which had been found that morning in the monkey house, were sent to the laboratory for examination and proved to be plague infected.

On visiting the gardens the same evening we ascertained that one of the rats had been found in cage I and the other in cage VI. The occupants of the different cages as we found them at this time are given in the table accompanying the diagram. It is to be noted that the monkey which inhabited cage I had changed places with the monkeys from cage II, and that the lemurs from cage VI had been removed to cage VII, the langurs from the latter cage taking their place.

The first plague death amongst the monkeys occurred on 17/4/06, when a black ape was found dead in cage IV. This was soon followed by the death of a Bonnet monkey in cage I on 19/4/06, a langur in cage VI on 20/4/06, another black ape in cage IV on 21/4/06, and a pig-tailed monkey in cage V on 27/4/06 (*vide* diagram).

It will be seen that the rats were found six days before the death of the first monkey and 16 days before the death of the last. Two more plague rats were found at a later date, namely, on 8/5/06, one in the passage outside cage I and the other in cage II on 10/5/06. Cage II was at this time inhabited by a single monkey, a crab-eating monkey, which did not contract the disease. It is noteworthy that in this small epidemic four species of monkeys became infected, viz.

- (1) Bonnet monkey (*Macacus sinicus*),
- (2) Black ape (*Cynopithecus niger*),
- (3) Langur (*Semnopithecus entellus*),
- (4) Pig-tailed monkey (*Macacus nemestrinus*).

It only remains to add that fowls, ducks, pigeons, goats, sheep, oxen, buffaloes and horses are common animals in Bombay, but that in no

single instance have we observed natural plague in any of them, nor do our observations lead us even to suspect that these animals play any part in the spread of the epidemic.

*Diagram of Monkey House and Table showing inhabitants.*

V 0 27/4/06	+ 11/4/06 VI 0 20/4/06*	VII	VIII
Pig-tailed Monkey		*Langur	
2 Black Apes			+ 8/5/06
0 17/4/06 IV 0 21/4/06	III	II + 10/5/06	I 0 19/4/06* + 11/4/06
			Bonnet Monkey
+ Plague-infected Rat.			
0 Plague-infected Monkey.			

*Inhabitants of cages on 11/4/06.*

- Cage I. 6 Bonnet Monkeys, *Macacus sinicus*, transferred from Cage II on 11/4/06.  
 Cage II. 1 Ourang-utang, *Simia satyrus*, transferred from Cage I on 11/4/06. Segregated on 19/4/06.  
 Cage III. 3 Baboons, *Cynocephalus hamadryus*.  
 Cage IV. 6 Black Apes, *Cynopithecus niger*.  
 Cage V. 1 Bonnet Monkey, *Macacus sinicus*. 2 Pig-tailed Monkeys, *Macacus nemestrinus*.  
 Cage VI. 3 Langurs. *Semnopithecus entellus*, transferred from Cage VII on 11/4/06.  
 Cage VII. 4 Lemurs, *Lemur macaco*, transferred from Cage VI on 11/4/06.  
 Cage VIII. 4 Malbronck Monkeys, *Cercopithecus cynosurus*.

## V. SUMMARY AND CONCLUSIONS.

(1) The question of the alleged spread of infection by direct contact with a suffering case has been discussed. Our observations in a plague hospital and with material obtained from this hospital lead us to conclude that such a mode of spread does not exist. Support is given to this view by a consideration of the influence of imported cases on the spread of the epidemic and by an investigation of the relative frequency of single and multiple cases in houses and buildings. We have, further, referred to our experience that a rat epizootic is alone sufficient to account for a widespread dissemination of infection throughout a

locality. A review of the whole of the evidence on this point brings us to the conclusion that contact with plague cases plays no part in the spread of the epidemic.

(2) In discussing the question of the infectivity of houses, evidence has been brought forward which points to the rat flea being the transmitting agent of infection from rat to man. Further, reasons have been given for the view that plague does not persist in a locality apart from infection amongst the rats.

(3) From arguments brought forward in the discussion of the two previous questions we conclude that the epidemic is wholly dependent upon the epizootics.

(4) It has been shown that infection may be transported to a distance by means of rat fleas in clothing or merchandise and that such infection, when imported into a hitherto uninfected locality, may give rise to an epizootic in the rats.

(5) Our observations lead us to conclude that plague in domestic animals in Bombay either does not occur or occurs so seldom that it cannot be said to possess any significance from an epidemiological standpoint.

## XXV. OBSERVATIONS IN THE PUNJAB VILLAGES OF DHAND AND KASEL.

- I. Introductory.
- II. Rats and fleas.
- III. Observations in Dhand village.
- IV. Observations in Kasel village.
- V. Experiments in plague houses.
- VI. Recurrence of plague in the same houses.

### I. INTRODUCTORY.

- I. Introduction.
  - (1) Situation of villages.
  - (2) Reasons for selecting these villages.
- II. Description of the villages.
  - (1) General description of a Punjab village.
  - (2) Construction of houses.
  - (3) Ventilation.
  - (4) Disposal of excreta.
  - (5) Circumstances in the houses favouring rats.
    - (a) Accessibility.
    - (b) Food-supply.
  - (6) Village officials.
  - (7) Census operations.
- III. Methods adopted for studying the epizootic and the epidemic.
  - (1) Observations on the rats.
    - (a) Live rats.
    - (b) Dead rats.
  - (2) Epidemic.
  - (3) Co-relation of epizootic and epidemic.

### I. INTRODUCTION.

The villages selected for the present series of observations were Dhand and Kasel in the Amritsar District of the Punjab. They are situated about 10 miles south-west of Amritsar City and within a distance of  $\frac{3}{4}$  mile of each other. They are typical Punjab villages of about 2,000 and 4,000 inhabitants respectively.



They were selected for the present observations for the following reasons:—

(a) Their comparative isolation and limited amount of communication with other villages and towns rendered it possible to follow the movements of the villagers and any visitors.

(b) Since plague first appeared in the Amritsar District in the spring of 1902, both the villages had suffered from three epidemics. The disease in the villages showed a marked seasonal prevalence, a period of rise and decline and subsequent complete disappearance, and this periodicity especially called for study.

(c) Finally, as the inhabitants in previous years had shown themselves, as compared with those of other villages, amenable to any plague measures suggested, it was thought that they would render assistance to the Commission.

## II. DESCRIPTION OF THE VILLAGES.

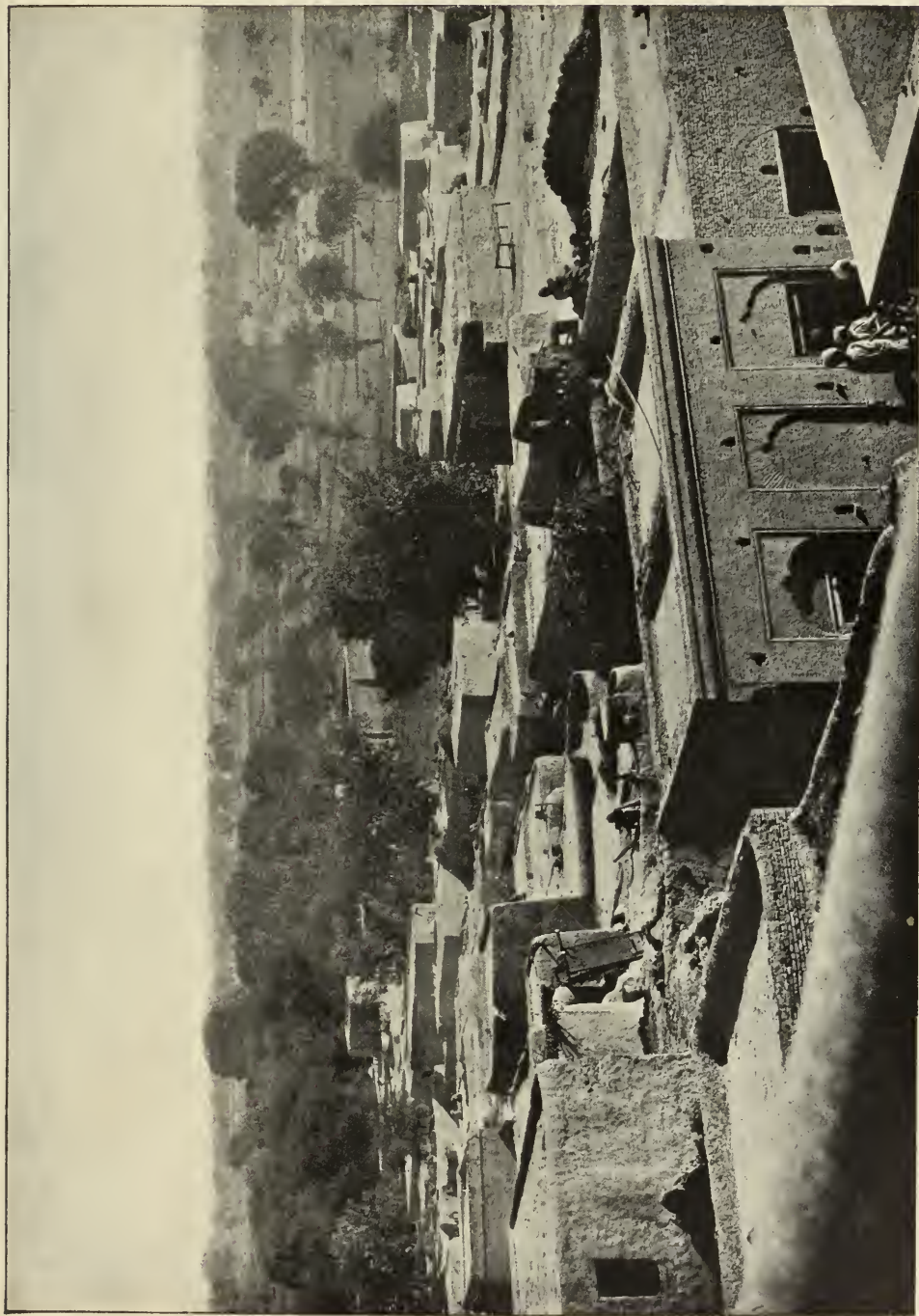
We propose to begin by giving a general description which applies to both villages and subsequently to give a special description and the census details for each village separately.

### 1. *General description.*

The following description is, with a few modifications and additions, taken from the excellent account of a Punjab village in Captain A. H. Bingley's book on Sikhs<sup>1</sup>.

The Punjab village (Plate XXXV) is almost always composed of houses built of sun-dried bricks or of large clods of caked-mud, taken from the bottom of a pond. But there are few villages which do not contain one or two fire-baked brick buildings (*vide* Plate XXXVI), the home of a well-to-do headman, of the village money-lender, or, perhaps, of a pensioned native officer. The houses, crowded as closely as they can be, are separated by narrow, winding lanes often only a few feet wide. Those of a "patti" or ward lie together and often have a separate entrance with a gateway. Between the actual buildings and the cultivated fields is an open space running right round the village, sometimes shaded by pipal trees and almost always in a very insanitary condition. Carts, which would take up too much room in the village stand there, and there it is that the cane press will be seen at work in the winter.

<sup>1</sup> *Handbooks for the Indian Army: Sikhs.* Simla, 1899.



Kasel village.





896



Kassel village.





89



Kasel village.





Kasel village.



546



Kasel village: interior of house with "kothi."







Kasel village: interior of house with "bharola" and "gharas."



At one or more sides of the village are ponds, from which earth has been excavated for the repair of houses and in which cattle are bathed and watered.

The backs of the houses are usually blank walls forming an outer boundary to the settlement.

In the space surrounding the village are found the manure heaps and stacks of cowdung fuel belonging to each household.

Entering the village we find the doorways of the houses opening on the main street or on side lanes running off them. Ordinarily these doorways lead straight into an open court yard, with cattle troughs along one or more of its sides. The dwelling-house proper will generally be found along the side of the courtyard which fronts the doorway.

The dwelling-houses are long and narrow, with or without a small verandah in front called a "dalan," and are generally provided at one side with a flight of stairs or a wooden ladder giving access to the roofs (Plate XXXVII). Windows there are none; light and air are admitted by the door, and the smoke finds its way out by the same way, or perhaps by a hole in the roof.

Cooking is carried out for the most part in a partially roofed shelter in a corner of the courtyard, for the people live as much as possible in the open air and are only driven indoors by cold or rain.

A noticeable feature in every house is the receptacles for grain, made of plastered mud. These vary in shape; the commonest type is an oblong cupboard (kothi), raised about a foot from the ground with a space of about six inches between it and the adjoining house wall and furnished with a small wooden door for putting in and removing the grain (Plate XXXIX).

Another form of corn-bin is a large jar-shaped receptacle (bharola) (Plate XL).

The families dwelling within one courtyard as a rule are related to one another and belong to the same caste.

Each family living within the courtyard has a separate dwelling-house and cooking place, while in the yard, outside the doors, much of the available space is taken up by the "charpoys" (string beds) and water-pots of the household and the spinning wheels and grindstones of the women. The roof is used for storing heaps of jowar (grain), fodder and bundles of cotton twigs which are used for roofing purposes, also for drying chillies, Indian corn, etc., in the sun.

Occasionally there is a small upper room (chaubara) on the roof (Plate XXXVIII), but this is rare in most villages.

Sometimes the front door, instead of leading directly into the court, leads into a lodge or "deorhi," out of which a smaller door, placed so that the interior of the yard cannot be seen from the street, leads into the yard itself. The "deorhi" serves as a cart-house, tool-shed and stable, and also as a lodging for such guests as are not sufficiently intimate to be taken into the house.

## 2. *Construction of the houses.*

To the above description it is only necessary to add some account of the construction of the houses.

The mud walls are from  $1\frac{1}{2}$  to 2 feet thick and in single storeyed houses average about 10 feet in height. Resting on the top of the walls are large rough-hewn beams ("shatir"), which support the roof (see Plate XXXIX). Lying across these beams are smaller rafters (karian), which in their turn support either bundles of cotton twigs or "sirki" (split cane), matting or cane.

The roof is completed by a layer of beaten earth from six to eight inches thick, which is immediately supported by the twigs, matting or cane. In the case of mud-built (kutchra) houses the walls are not sunk, but in brick and masonry houses they extend to a depth of from one to seven feet below the ground level. In both classes of houses the floor consists merely of beaten earth, which may or may not be plastered with cowdung (leaped).

Only very exceptionally is the floor raised above the general ground level. The upper storey, when it exists, often consists of a single room built on the middle of the roof and furnished with windows on all four sides (hence the Punjabi name for an upper story, "chaubara," meaning four entrances). These upper rooms thus afford a striking contrast in the matter of ventilation to the rooms on the ground floor.

## 3. *Ventilation.*

It will be apparent from the above description of the village house that, except in the upper storeys, no means for ventilation, except the doors, exist. The villagers sleep indoors from November to March with closed doors during the colder months, and the vitiation of the atmosphere is obvious on entering a house in the early morning.

Excluding storerooms and rooms for cattle, and taking into account only rooms actually occupied, we found the number of square feet of floor space per occupant to be in Dhand 50 and in Kasel 40. These



figures compare favourably with those for Bombay and other large Indian cities.

4. *Disposal of excreta.*

The adult inhabitants resort to the fields outside the village for purposes of defecation. Young children, and adults confined to their houses by illness, pass their excreta into an earthen vessel or on to the actual floor of their houses or court yards. In either case the faeces are removed outside the village by sweepers.

5. *Circumstances in the houses favouring rats.*

These may be considered under the headings of (a) accessibility; (b) food-supply.

(a) *Accessibility.* We have seen that the large majority of village houses are built either of sun-dried bricks or of large clods of caked-mud. These mud walls, as well as the mud floors and roofs, present no obstacle to the rat, which burrows freely in all three.

(b) *Food-supply.* As mentioned above every Punjab house contains its granary (kothi, bharola, etc.). The well-to-do cultivator, after the spring harvest, stores sufficient wheat for the needs of his household for the entire year in his granaries, cellars and storerooms, while the Kamin classes receive in return for their services several months' supply. In all the houses there is thus abundant grain kept in places more or less accessible to rats. In addition to wheat, which is the staple foodstuff of the villagers, numerous other varieties of grain, flour, sugar, ghee (clarified butter), potatoes, onions, etc. are kept in earthen vessels (gharas), of which large numbers are to be seen in every village house (*vide* Plate XL). On several occasions we have received from villagers rats which have been caught alive in these vessels, where they had gone in search of food.

6. *Village officials.*

As in the description of the methods adopted for studying plague in these villages we shall have to refer to the various agencies which we utilised for obtaining information about plague rats and cases, it is necessary here to give a short description of the village officials who gave us assistance.

The Zaihlidar is a native official in charge of a collection of six to ten villages, termed a Zaihl. He is responsible for the collection of the

revenue in his Zaihl and the carrying out of all orders, sanitary or otherwise, emanating from the Tahsil<sup>1</sup>.

Under the Zaihldar are two or more officials, termed "Safed Posh," each of whom is directly in charge of a subdivision of the Zaihl, consisting of a group of three or more villages.

The Zaihldar and Safed Posh are head lumbardars of their own villages, and we were fortunate in having these two influential persons residing the one in Kasel and the other in Dhand.

Coming to the individual village we find that each is subdivided into two or more "pattis" or wards. Each ward is in charge of a recognised official called a "lumbardar," the head lumbardar of a village being termed the "ala lumbardar." Each lumbardar is responsible for the collection of the land revenue and hearth-tax in his own patti.

They are assisted in their duties by the village "chowkidars." The latter (chowkidars) are village menials, usually Mahomedans of a caste called Barwalas. There is one to each "patti." Their chief duties are to report cases of crime to the nearest police station, to report births and deaths in their respective pattis to the lumbardars who enter them in their village registers and to assist the latter in collecting revenue.

Lastly, we have to refer to the village "Patwaris." These officials are paid by Government to keep accounts of land and revenue. They are trained in survey work and proved most useful in making maps of the villages to scale. Their thorough knowledge of the inhabitants and their relatively high standard of education and intelligence enabled them to be of great assistance to us in making a census of the village.

It will be evident from the above description of the village officials, that there already existed in both villages an agency, capable of exercising a very complete supervision over the inhabitants; and through the kindness of the Civil authorities we were permitted to make full use of it.

#### 7. *Census operations.*

The first step taken was to number every house. For this purpose we defined a house as a domicile occupied by a family which had a common cooking place. For instance, when, as commonly happens, several brothers and their families occupy adjoining houses in the same courtyard and each family cooks separately, each house received a separate number.

<sup>1</sup> A District in the Punjab for administrative purposes is divided into Tahsils, each of which, as we have seen, is made up of Zaihlis.

For convenience of reference, numbers were also given to stables, shops and unoccupied houses, which strictly speaking do not come within the above definition of a house.

Having completed the numbering of the houses, a detailed map to scale (100 feet to 1 inch) of each village was prepared by the Patwaris. These maps showed every house with its number. A complete census of each village was then made, full particulars for each house being recorded on special "Census Cards." These particulars included the name, age, sex, caste and occupation of every inhabitant.

Further, an estimation of the amount of space available per occupant was made for each house. Probable sources of attraction for rats, such as the proximity of cattle sheds or grain and chaff stores to the dwelling-houses, were noted.

Finally, with a view of ascertaining how far plague tends to recur in particular houses full particulars of the previous incidence of plague in each house were obtained and noted on the cards.

### III. METHODS ADOPTED FOR STUDYING THE EPIZOOTIC AND EPIDEMIC.

#### 1. *Observations on the rats.*

##### (a) *Live rats.*

A systematic examination of the rats in the villages was carried out. Every evening numbered traps were set by the village chowkidars in different houses, the census numbers of the houses being, as far as possible, followed consecutively. The traps were removed in the morning under the immediate supervision of a Hospital Assistant, who made out a list of the trap numbers and the corresponding house numbers.

On arrival of the traps at the laboratory a count of the fleas on the rats was made with as little delay as possible. The traps were placed in pairs in tin-lined boxes with closely fitting lids and containing two compartments, each fitted with a removable tray on which the trap rested. Chloroform was shaken over the traps and the boxes were closed. After a sufficient interval the traps and trays were removed, and the fleas which had fallen on the tray were counted as well as those adhering to the fur of the dead rats. The total number of fleas found on the rat or rats in each cage was thus obtained and was noted in a special register. They were examined as regards the species to which they belonged, and a note of any *Ceratophyllus fasciatus* found was made. During several months of the period over which the observations extended an attempt was made to obtain a count of the relative numbers

of fleas found on different anatomical regions of the rat, viz. (a) the head and neck, (b) chest, axilla and fore-legs, and (c) abdomen, groins and hind-legs. For this count we could, of course, only take into consideration the fleas which were found on the rats after their removal from the chloroform box.

After the fleas had been removed and counted the rats were weighed and a label was attached to each. On this label the serial number, the date, the number of the house in which the rat was caught and the weight of the rat were noted.

The rats were then pinned out (*vide* Plate XLI) for dissection. The post-mortem examination was carried out in the way which has already been described for the Bombay rats. The cutting-up was done by two Hospital Assistants, trained to recognise the post-mortem appearances of plague rats. Every rat dissected was examined by a Member of the Commission, who also made a microscopical examination of smears from the spleen of every rat.

Animal and cultural tests were used when the diagnosis was uncertain. The results of this examination, including particulars as to sex, pregnancy and infection with plague, were now entered on the label.

At the conclusion of each day's work the details noted on the labels were copied into a rat register in serial order. In the case of plague-infected rats the result of the post-mortem examination and the other details were recorded on the cards ("plague rat cards"), which were used in Bombay and which have been already described.

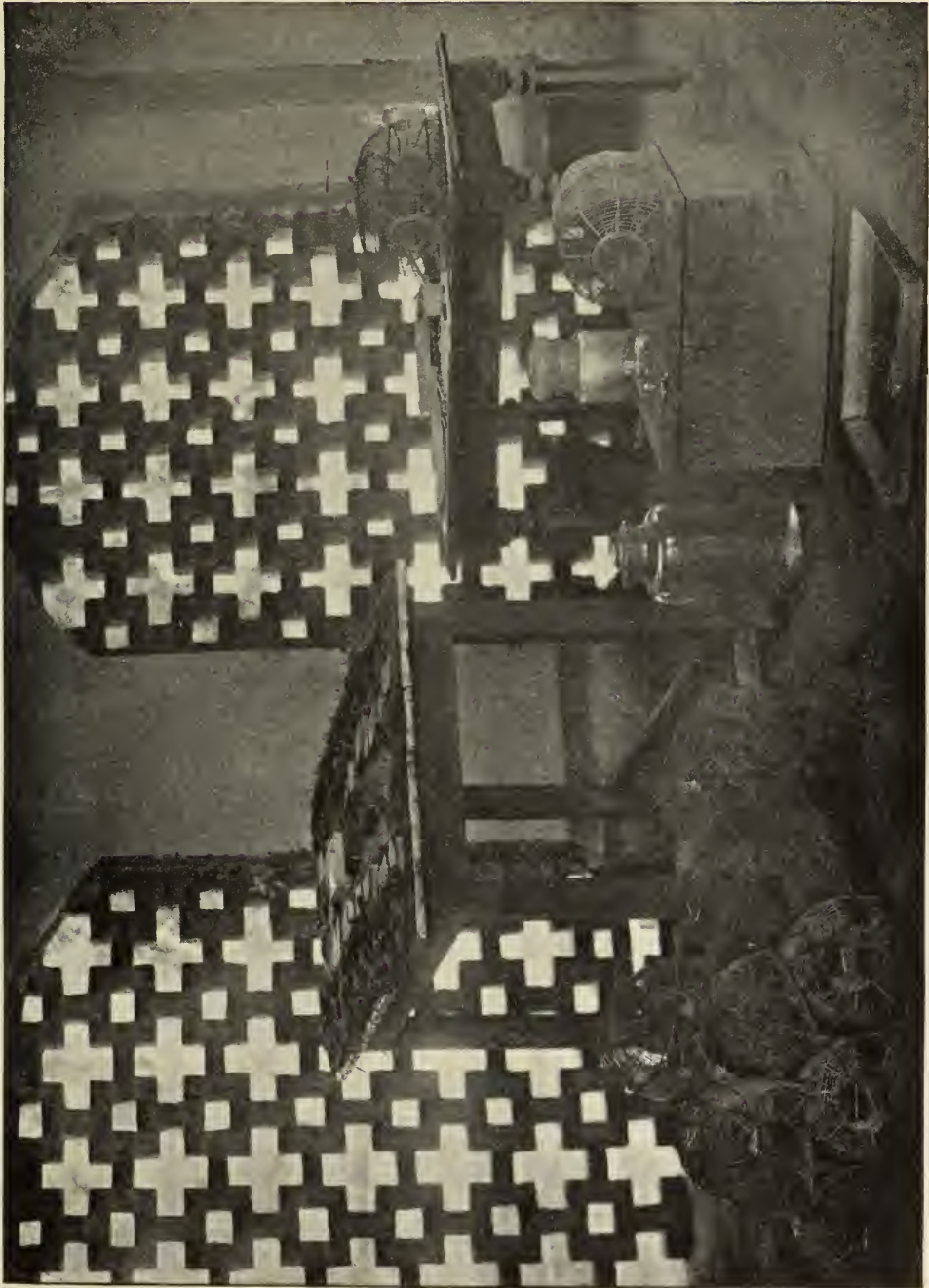
(b) *Dead rats.*

While systematic trapping was being carried out in the way detailed above, we made every effort to secure all the dead rats that were to be found in the village. The villagers were advised through the lumbardars to send us any dead rats they might find, and we promised to let them know whether they proved to be plague infected or not, so that in the event of the animals proving plague infected they might take precautionary measures, such as evacuation of their houses or inoculation. It was, however, carefully explained to them that under no circumstances would they be compelled to undergo inoculation or have their houses disinfected nor would they be compelled to evacuate them. The chowkidars made daily inquiries for dead rats in their respective pattis. During the epizootic they were provided with tins with closely fitting lids, in which they brought all dead rats to the laboratory.

The post-mortem examination of the dead rats and other details were recorded in the same way as was done in the case of the live rats.



90°



The Punjab Laboratory.





2. *The epidemic.*

It was realised that, unless we obtained the confidence of the people before the epidemic broke out, concealment of plague cases would certainly occur. It was, therefore, decided to open a small free dispensary at the laboratory, which the villagers were encouraged to attend. This measure met with considerable success, the daily attendance soon averaging from 15 to 20.

Further, all cases of illness about which we received information were visited and treatment was offered. As a result the people had grown quite accustomed to our presence among them by the time the epidemic broke out and concealment of plague cases was quite exceptional. When the relations of the patient did not themselves report the case to the chowkidar or lumbardar of their patti, information was usually quickly received from the neighbours.

At first it was intended that an attempt should be made to confirm the diagnosis of all cases by bacteriological methods, and this was actually done for the first few cases which occurred in Dhand. It was soon recognised, however, that any attempt to obtain material for examination as a routine measure would defeat its own object by leading to concealment of cases, as the patients and their relatives often naturally objected to the somewhat painful operation of puncturing the bubo in its early stage, though they were quite ready to have it incised when pus had formed. This procedure was, therefore, abandoned and the diagnosis of most of the cases was based on the clinical features, which were usually sufficiently typical. In a few instances where the patients were adult females examination of the inguinal regions for buboes was not permitted, and we had to rely on the statements of the patient and her friends.

3. *Co-relation of epizootic and epidemic.*

The details of all plague rats were entered at the laboratory on the special "plague rat" cards already referred to. On the same evening or the next morning these cards were taken to the village, where the addresses already noted on them were checked by a Member of the Commission, and all further particulars were entered in the cards under their appropriate headings. After the addresses had been checked each plague rat was marked in its proper position on the map and the date affixed. Similarly, every human case of plague reported was seen as soon as possible and, if diagnosed to be plague, full particulars

were noted on a "Human case card," which was of the same pattern as that used in Bombay. Special attention was directed to ascertaining the probable place of infection, where this was not obviously the place of residence. The plague cases, like the plague rats, were entered on the map with the date of attack. When the probable place of infection was other than the place of residence the case was shown by a different symbol in both places.

## II. RATS AND FLEAS.

### I. Observations on the rat population.

- (1) Remarks on the animals examined.
- (2) Observations in connection with *Mus rattus*.
  - (a) Species.
  - (b) Habits.
  - (c) Breeding.
  - (d) Migration.
  - (e) Normal mortality.
  - (f) Post-mortem appearance of plague rats.
  - (g) Diseases other than plague among rats.
  - (h) Rat infestation of the villages.

### II. Observations on the fleas infesting the rats.

#### I. OBSERVATIONS ON THE RAT POPULATION.

##### 1. *General remarks on the animals examined.*

During the period during which the observations continued, namely, from 29th November 1905 to 30th November 1906, the total number of *Mus rattus* examined from Dhand and Kasel was 7525, of which 7164 were trapped alive and 361 were delivered dead at the laboratory. Plague was identified in 52 live and 290 dead rats. No *Mus decumanus* were found.

In addition to the above rodents the following animals were examined:—5 mice, 19 musk rats (*Crocidura coerulea*), 9 gerbils (*Gerbillus indicus*) and 25 *Nesokia bengalensis*. Out of these one mouse and one musk rat were found infected with plague. The musk rat was found dead in Kasel during the epizootic. All the musk rats and 7 of the gerbils were obtained in the villages, most of the latter from houses on the outskirts. The two remaining gerbils and all the *Nesokia* were taken in the fields at harvest time. The absence of *Nesokia* in the interior of the villages is noteworthy, as this species

appears to be common in Calcutta and by no means rare in Bombay City.

The small number of mice caught may in part be accounted for by the traps used being unsuitable for catching this species. Inquiries in the villages, however, appear to show that mice are not commonly seen in houses.

2. *Observations in connection with Mus rattus.*

(a) *Species.* The rats taken in the Punjab villages were all of one species, namely *Mus rattus*. The type resembles very closely that found in Bombay, which has been already described. The colour of the dorsal fur is usually brown, while the belly is greyish, dirty-yellow or occasionally quite white. The fur during the cold weather is longer and thicker than that of the *Mus rattus* found in Bombay.

(b) *Habits.* *Mus rattus* in the Punjab, as in Bombay, is essentially a house rat. We have seen that the houses in a Punjab village are well adapted for harbouring this animal, both on account of the abundant grain supply and on account of the facilities for burrowing which the mud walls and floors offer. It is indeed the exception to find a village house which does not show evidence of rat infestation, such as rat holes and rats' dung. In illustration of the close relation that exists between rat and man, it may be mentioned that a common complaint of the villagers is that their sleep has been disturbed by rats running over them at night.

In contrast with *Mus rattus* in Bombay city, the Punjab rat burrows extensively. In this respect he resembles *Mus decumanus* in Bombay, as also in the peculiarity that his nests are always found in the burrows. We have never found rats' nests actually in the houses, *i.e.* among rubbish collected in the rooms. This is a situation in which rats' nests are commonly found in Bombay. It is almost certain that the rat-burrows in the Punjab villages are very extensive, ramifying beneath and opening up communication between several contiguous houses. On two occasions we have actually traced burrows in the walls of houses which were in process of being pulled down. These burrows, which contained mummified rats, extended the entire length of the wall, as far as we could open it up and into the wall of an adjoining house, where we could not follow them.

(c) *Breeding.* An attempt was made to ascertain if there is any special breeding season of *Mus rattus* in the Punjab. The same method was adopted as was used in Bombay. The number of pregnant females

and the number of young (70 grammes and under) rats were recorded each day. The percentage of pregnant females to adult females and of young rats to total rats was calculated for each month. The results are given in Tables I and II, in which the figures for Dhand and Kasel are combined. The figures showing the percentage of pregnant females among adult females refer to live rats only, while the corresponding figures for young rats include both dead and live rats (see also chart 1).

TABLE I.

*Showing percentage of pregnant females among total adult females—  
Dhand and Kasel.*

*Mus rattus*—alive.

Month	Total adult live females	Total adult pregnant females	Percentage of pregnant to total adult females
December	668	126	18·9
January	293	71	24·2
February	266	70	26·3
March	461	154	33·4
April	145	73	50·3
May	142	49	34·5
June	94	34	36·2
July	50	20	40·0
August	84	34	40·5
September	152	75	49·3
October	131	66	50·4
November	334	105	31·4

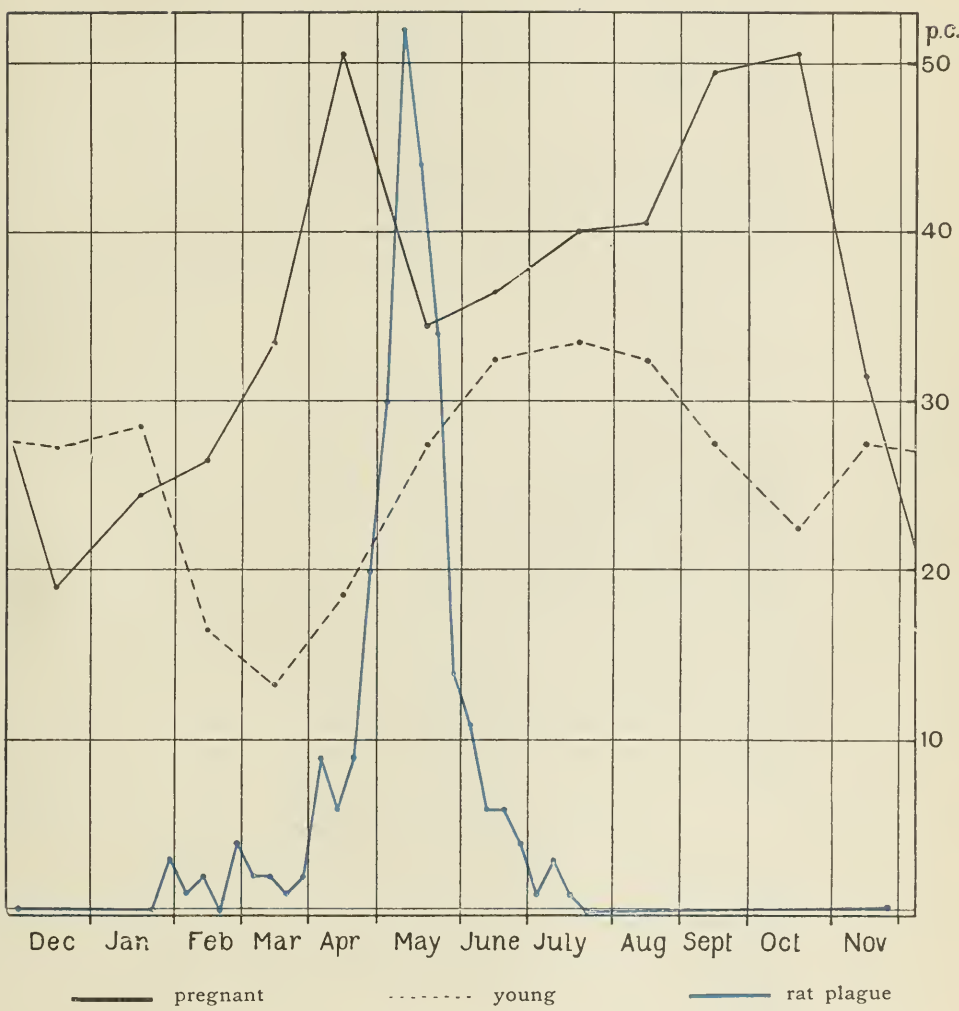
TABLE II.

*Showing percentage of young rats among total rats—Dhand and Kasel.*

*Mus rattus*—live and dead.

Month	Total rats alive and dead	Total young rats alive and dead	Percentage of young rats on total rats
December	1842	503	27·3
January	824	234	28·4
February	670	108	16·1
March	1037	136	13·1
April	408	76	18·6
May	486	132	27·2
June	259	84	32·4
July	143	48	33·6
August	234	76	32·5
September	373	102	27·3
October	370	83	22·4
November	879	241	27·4





Breeding of *Mus rattus* in Kasel and Dhand



From the tables and the chart it will be seen that breeding goes on all the year round. The lowest percentage of pregnant females among adult females was about 19 in December and the highest about 50 in April, September and October, the mean percentage for the whole period being 31. The curve of pregnant females shows a maximum in April and again in September and October. In this connection it may be remarked that the mean temperature of April does not differ much from that of September and October.

Interpreting the curve broadly, we may say that during the cold weather months (November to March inclusive) breeding takes place to a less extent than during the hot weather, and that the most favourable months are April, September and October, when the mean temperature approximates to 80° F.

The curve of young rats follows as closely as could be expected the pregnant female curve.

The number of foetuses in utero was counted in 975 pregnant rats, the average number found being 6 per rat.

(d) *Migration*. An attempt was made to determine by direct experiment whether any migration of rats from villages takes place. With this object traps were placed during January and part of February outside Kasel village, at distances varying from a few yards to two hundred yards from the houses. In all 940 traps were set, and in one instance only, when the traps were placed closed to a house, were a few *Mus rattus* caught. When trapping was resumed in Kasel towards the end of February, we were obliged to discontinue the trapping outside the village. The above experiment is not conclusive, as it was done during the cold weather and at a time when there was no plague amongst the rats in the village. It is, therefore, probable that at this time there would be little tendency of the rats to migrate.

The statement has been made by some workers on plague, that at the time the harvest is cut (April—June), rats leave the village and go into the fields, and on this basis an attempt has been made to explain the seasonal decline of the epidemic. We may say that we failed to obtain any evidence that such a migration takes place. The only animals at all resembling *Mus rattus* which we found in the fields during the harvesting of the spring crops in April, May and June, were specimens of *Nesokia bengalensis*. The superficial resemblance of this rodent to *Mus rattus* probably accounts for the idea of some of the farmers that rats migrate from the villages to the fields. On the other hand, the more intelligent cultivators recognise the distinction between

these two species and emphatically deny that rats ever leave the village. It may be mentioned that the epizootic in Kasel coincided in point of time with the harvest months.

(e) *Normal mortality.* Table III shows the number of rats found dead in the villages from causes other than plague. In addition to rats proved to be plague infected, all putrid rats found during the epizootic period, in which plague was not negatived, are excluded. It will be seen that only 116 such dead rats were found during the year. It seems certain that this number cannot represent the normal rat mortality and it may, therefore, be inferred that the large majority of rats which die from normal causes die in inaccessible places.

TABLE III.

*Showing the number and post-mortem appearances of rats found dead in Dhand and Kasel from causes other than plague during the 12 months ending 30th November 1906.*

Post-mortem appearances	No. of rats
Nothing naked eye or on microscopic examination	57
Signs of injury	26
Putrid but not proved by animal test to be plague infected	15
Oedema of neck	1
Abscess of lung	1
Tumours	1
Granular spleen or liver; subcutaneous congestion; pleural or peritoneal effusion; or other appearances suggesting death from plague, but not confirmed by animal tests	15
Total	116

NOTE:—A considerable number of these rats were found infected with trypanosomes.

The interesting question arises, whether the number of plague rats found above ground bears a similarly small proportion to the total number which die of plague. During the epizootic period 226 dead plague rats were found in Kasel, while during the same period only 23 rats which had died from other causes were discovered.

Assuming that the chances of finding dead plague rats and non-plague rats are equal, it might be argued that during the epizootic the rat mortality from plague in Kasel was 10 times the normal mortality.

It was impossible to extend the search for plague rats into the burrows, as this would have involved much damage to the houses. Hence we cannot bring forward any direct evidence as to the number

which die in their burrows. However, on the two occasions on which we had the opportunity of opening up rat burrows in the walls, mummified carcasses of rats were found in them, and the villagers say that they are very commonly found when houses are being pulled down.

(f) The post-mortem findings in acute plague rats in Bombay have been already described (vol. VII., p. 324): the results obtained in the Punjab were the same (see Tables IV, V, VI). The morbid anatomy of the chronic plague rats in the Punjab has been detailed above (vol. VII., p. 457).

TABLE IV.

*Showing the frequency of the common post-mortem signs in the acute plague rats found during the epizootic in Kasel (252) and Dhand (34).*

	Kasel	Dhand
Subcutaneous congestion	142 (56 p.c.)	19 (56 p.c.)
Granular liver	63 (25 p.c.)	17 (50 p.c.)
Granular spleen	43 (17 p.c.)	9 (26·5 p.c.)
Pleural effusion	191 (76 p.c.)	17 (50 p.c.)
Haemorrhages, subcutaneous and elsewhere	176 (70 p.c.)	18 (53 p.c.)

TABLE V.

*Showing the analysis of the results of the microscopical examination of the bubo, spleen, and heart's blood of the acute plague rats found during the epizootic.*

		Dhand	Kasel
Bubo	No <i>B. pestis</i>	2 (11·1 p.c.)	11 (7·1 p.c.)
	Few „	4 (22·2 p.c.)	12 (7·8 p.c.)
	Numerous „	12 (66·7 p.c.)	113 (73·4 p.c.)
	Involution forms	0	18 (11·7 p.c.)
Spleen	No <i>B. pestis</i>	13 (38 p.c.)	31 (12·4 p.c.)
	Few „	2 (6 p.c.)	6 (2·4 p.c.)
	Numerous „	18 (53 p.c.)	184 (73·6 p.c.)
	Involution forms	1 (3 p.c.)	29 (11·5 p.c.)
Heart's blood	No <i>B. pestis</i>	11 (39·3 p.c.)	64 (26·5 p.c.)
	Few „	3 (10·7 p.c.)	26 (10·7 p.c.)
	Numerous „	14 (50 p.c.)	149 (61·6 p.c.)
	Involution forms	0	3 (1·2 p.c.)

(g) *Diseases, other than plague, amongst the rats.* Apart from plague the commonest pathological conditions found in Punjab rats were peripheral abscesses. The organism most frequently isolated from these abscesses was a very small diplo-bacillus, which gave a feeble growth on agar and usually could not be subcultured. This bacillus was non-pathogenic to guinea-pigs. As about 60% of these abscesses





were found in connection with superficial lymphatic glands, it seemed possible that they might have originated as plague buboes and have undergone a secondary infection with the bacillus above described. It was found, however, that the distribution of these glandular abscesses differed somewhat from that of plague buboes found in Punjab rats, as may be seen by reference to Table VII.

TABLE VII.

*Comparison of distribution of buboes in plague rats and of chronic abscesses occurring in situation of lymphatic glands but not containing B. pestis.*

Situation	Plague buboes		Chronic abscesses not containing <i>B. pestis</i>	
	number	percentage	number	percentage
1. Single	150	86	81	100
a. Submaxillary	81	54	42	52
b. Axillary	36	24	7	9
c. Inguinal	30	20	30	37
d. Pelvic	3	2	2	2
2. Multiple	23	14	0	0

TABLE VIII.

*Showing percentage of Mus rattus found infected with Adie's Leucocytozoon, each month.*

Month	No. of rats examined	No. of rats infected	Percentage of rats infected
December 1905	1823	306	16·8
January 1906	843	123	14·6
February	707	50	7·1
March	1051	43	4·1
April	451	3	0·7
May	574	31	5·4
June	352	79	22·4
July	598	130	21·7
August	505	109	21·6
September	504	97	19·2
October	879	147	16·7
November	1212	191	15·8
Total	9499	1309	13·8

The other diseases met with include a very few cases of abscess of the lung, abscess of the liver, "acid fast" disease, ovarian and other abdominal tumours. Trypanosomes were commonly found, especially in young rats. Another common blood parasite found was the leucocytozoon recently described by Adie (*Journal of Tropical Medicine*, 1906,

vol. IX. p. 325). A daily record of the number of rats in the spleen of which this parasite was found was kept. At the end of the observations it was found that the percentage of rats infected for the entire year was 14. Table VIII shows the percentage found infected during each month of the year. We have no reason to suppose that this parasite is pathogenic.

(h) *Rat infestation of the villages.* Tables IX and X show the extent of the rat infestation of the two villages during each month, as indicated by the number of rats taken per 100 traps. It will be seen that in Kasel the total number of *Mus rattus* trapped in the complete year was 4639 or an average of 1.2 rats per inhabitant. The corresponding figures for Dhand are 2518 and 1.3 per inhabitant. In Kasel the majority of houses were trapped 10 times and in Dhand 17 times during the year.

TABLE IX.

*Showing the results of rat trapping in Dhand month by month.*

Month	No. of traps set	No. of rats taken	Average No. of rats per 100 traps
December 1905	943	742	80
January 1906	2393	801	34
February	1699	258	15
March	1492	141	10
April	436	21	5
May	534	17	3
June	936	28	3
July	—	—	—
August	—	—	—
September (14 to 30)	233	78	34
October	301	149	50
November	875	283	32
Entire year	9842	2518	26

It is somewhat difficult to compare the relative extent of rat infestation of the two villages. A reference to the tables shows that the average number of rats caught per 100 traps for the whole year was 54 in Kasel as compared with 26 in Dhand. But if we seek to conclude from these figures that the rat infestation of Kasel was originally double that of Dhand, we are met by the objection that the relatively heavier trapping in Dhand throughout the year must have tended to reduce the average number of rats taken per 100 traps for the whole period, as, other factors being equal, the catch must have diminished with each successive trapping. For the same reason we cannot express the relative

rat infestation of the two villages in terms of the number of rats taken per inhabitant. On the whole the best approximation would seem to be obtained by comparing the number of rats taken per 100 traps in each village during the first complete trapping. These numbers are for Kasel 148 rats per 100 traps and for Dhand 96, from which we infer that at the time we commenced the observations in the villages the rat infestation of Kasel was about 50 % higher than that of Dhand.

TABLE X.

*Showing the results of rat trapping in Kasel, month by month.*

Month	No. of traps set	No. of rats taken	Average No. of rats per 100 traps
December 1905	782	1080	138
January 1906	—	—	—
February (20 to 28)	448	398	89
March	1260	870	69
April	586	316	54
May	927	315	34
June	1048	202	19
July	772	132	17
August	774	232	30
September	619	292	47
October	531	215	40
November	854	587	69
Entire year	8601	4639	54

Tables IX and X further show that for several months after trapping was commenced its effect was to reduce progressively the number of rats caught. Later, however, although trapping was continued, the numbers caught began steadily to increase, showing that breeding was able to more than compensate for the loss sustained by trapping. The effect of the plague epizootic on the number of the rat population will be discussed fully in a later paper on the seasonal prevalence of plague.

During November 1906 in Kasel the average number of rats caught per 100 traps set was 69 or exactly half the corresponding number for December 1905. In other words, the apparent rat population had been reduced by one half during the year. Assuming that the rat population of the village for any given months in the off-plague season remains fairly constant from year to year (breeding and mortality from all causes balancing each other), this reduction of the rat population may be taken to be due to trapping. The total number of rats trapped

was roughly 5000, and we have seen that the removal of this number reduced the apparent rat population to about one half. Hence, we can roughly estimate the original rat population at 10,000. On a similar estimate the rat population of Dhand would be originally about 4000. These figures would not include rats under 10 grammes weight, as we have not trapped any below this weight.

Table XI shows the rat infestation of Kasel as a whole and the relative infestation of different classes of houses, etc. The average number of rats taken per occupied house (*i.e.* occupied by a single family) for the entire period was 5·2, and the number of rats per 100 traps sets for the same class of houses 53·8. An analysis of the figures for 21 "pukka" (brick and mortar) houses shows that they do not differ appreciably from "kutcha" (mud) houses as regards rat infestation. It is to be noted, however, that the brick and mortar houses in the village resemble the other (kutcha) houses in having floors of beaten earth. As might have been expected from the abundant supply of grain which they contain in accessible situations, shops show a high degree of rat infestation. The degree of infestation of stables, unoccupied houses and godowns is relatively low.

TABLE XI.

*Showing rat infestation of Kasel as a whole and the relative infestation of different classes of houses, stables, etc.*

	Description of house, etc.	No. of houses trapped	No. of traps set	No. of rats caught	Average No. of rats per 100 traps	Average No. of rats per house, stable, etc.
1	All kinds	1084	8642	4711	54·5	4·3
2	Occupied dwelling houses (all kinds)	783	7545	4059	53·8	5·2
3	Occupied dwelling houses (Pukka masonry)	21	290	142	50·0	5·8
4	Stables	137	416	149	35·8	1
5	Unoccupied houses	83	307	137	44·6	1·7
6	Shops	49	260	321	123·5	6·6
7	Godowns	32	114	45	39·5	1·4

## II. OBSERVATIONS ON THE FLEAS INFESTING THE RATS.

The bionomics and the anatomy, both external and internal, of the fleas found in association with rats in India are dealt with in other papers. It will be sufficient here to briefly mention the species of fleas found on Punjab rats and the seasonal prevalence of each species.

1. *Pulex cheopis*. In the Punjab, as in Bombay, the common rat flea is *Pulex cheopis*, 98% of the fleas found on *Mus rattus* belonging



to this species. We have also commonly found this flea on musk rats and occasionally on gerbils caught in the village houses.

Tables XII and XIII show the average number of fleas of this species per rat caught for each month from December 1905 to November 1906 in Dhand and in Kasel. It will be seen that the maximum flea prevalence in Dhand was in February and in Kasel during April. It is

TABLE XII.

*Showing average number of fleas (P. cheopis) per rat month by month in Dhand.*

Month	Total No. of live rats on which fleas were recorded	Total No. of fleas	Average No. of fleas per rat
December 1905	704	5037	7.2
January 1906	803	9025	11.2
February	258	3220	12.5
March	141	1302	9.2
April	20	251	12.5
May	17	100	5.9
June	28	95	3.4
July	—	—	—
August	—	—	—
September	78	157	2.0
October	152	751	5.0
November	281	2633	9.4
Total	2482	22571	9.1

TABLE XIII.

*Showing average number of fleas (P. cheopis) per rat month by month in Kasel.*

Month	Total No. of live rats on which fleas were recorded	Total No. of fleas	Average No. of fleas per rat
December 1905	927	6987	7.5
January 1906	6	60	10.0
February	389	3038	7.8
March	879	6767	7.7
April	330	4145	12.6
May	324	2382	7.4
June	204	1036	5.1
July	136	514	3.8
August	226	447	2.0
September	296	696	2.4
October	223	1204	5.4
November	586	4147	7.1
Total	4526	31423	6.9

remarkable that February and April were the first months of the epizootics in Dhand and Kasel respectively. Owing, however, to the small numbers of rats received from Dhand from April to August inclusive and from Kasel during January and part of February, it is not possible to compare the seasonal flea prevalence in the two villages. For the same reasons we are perhaps not justified in considering that the months of maximum flea prevalence really differ in the two villages as the February figures for Kasel are incomplete and the April figures for Dhand are calculated on 21 rats only.

Table XI and Chart 2 which show the figures of the two villages combined may, however, be considered to fairly represent the variations in flea prevalence from month to month. From a study of this chart and table we may, without laying too much stress on minor variations, draw certain general conclusions. Thus the number of rat fleas is above the average from November to May with a maximum probably in April. During the remaining months, June to September, the flea prevalence is below the average, the absolute minimum being reached in August and September, when the number per rat is 6 times less than in April.

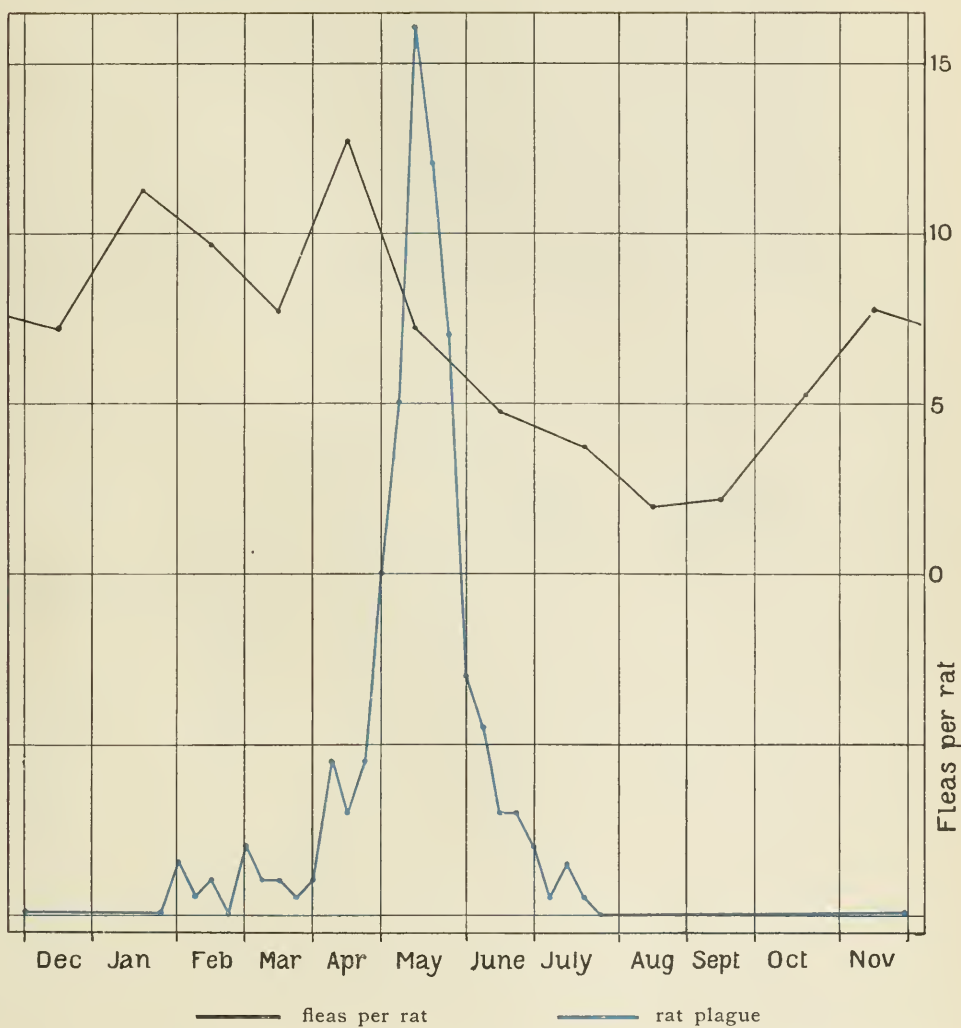
The influence of this seasonal variation of fleas on the seasonal prevalence of plague will be fully dealt with in another paper.

TABLE XIV.

*Showing the average number of fleas (P. cheopis) per rat month by month in Dhand and Kasel.*

Month	Total No. of live rats on which fleas were recorded	Total No. of fleas	Average No. of fleas per rat
December 1905	1631	12024	7.4
January 1906	809	9085	11.2
February	647	6258	9.7
March	1020	8069	7.9
April	350	4396	12.6
May	341	2482	7.3
June	232	1131	4.9
July	136	514	3.8
August	226	447	2.0
September	374	853	2.3
October	375	1955	5.2
November	867	6780	7.8
Total	7008	53994	7.7

2. *Ceratophyllus fuscatus*. About 2% of all the fleas taken on Punjab rats belong to this species. This flea has a very definite



Prevalence of *Pulex cheopis* in Kasel and Dhand



seasonal prevalence in the villages investigated. It was found to be present on the rats in both villages when we commenced observations in the beginning of December 1905. It disappeared from Dhand about the end of March and from Kasel about the middle of April, except for an isolated specimen found in May. From this date no fleas of this species were found till the first week in November, when they reappeared almost simultaneously in both villages, and until trapping was stopped in the first week in December they remained present. Table XV shows the number of *Ceratophyllus* caught during each month and their percentage of total fleas. It is evident that the season during which this flea is found on rats extends from November to April. We have seen already that *Pulex cheopis* also is most prevalent during these months.

TABLE XV.

*Showing prevalence of Ceratophyllus fasciatus in Dhand and Kasel for each month.*

Month	No. of <i>C. fasciatus</i>		Percentage on all fleas		Average No. per live rat.	
	Kasel	Dhand	Kasel	Dhand	Kasel	Dhand
December 1905	record	record	—	—	—	—
	not complete	not complete	—	—	—	—
January 1906	village					
	not trapped	298	—	3·4	—	0·4
February	82	70	2·7	2·2	0·2	0·3
March	289	35	4·3	2·7	0·35	0·25
April	14	—	0·3	—	0·04	—
May	1	—	—	—	—	—
June	—	—	—	—	—	—
July	—	—	—	—	—	—
August	—	—	—	—	—	—
September	—	—	—	—	—	—
October	—	—	—	—	—	—
November	112	11	2·7	0·5	0·2	0·04
December	17	—	2·5	—	0·2	—



### III. OBSERVATIONS WHICH HAVE SPECIAL REFERENCE TO THE VILLAGE OF DHAND.

#### I. Introduction.

- (1) Situation, etc.
- (2) Census results.

#### II. Previous epidemics of plague.

#### III. The plague epidemic of 1906.

- (1) Period before the epidemic.
- (2) Period during the epidemic.
  - (a) Severity and duration of the epidemic.
  - (b) Clinical features of cases.
    1. Nature of the cases.
    2. Situation of the primary bubo.
    3. Mode of onset.
    4. Sex incidence.
    5. Age incidence.
    6. Caste incidence.
    7. Case mortality.
  - (c) Distribution of the cases amongst the houses.
  - (d) Contact with previous cases.

#### IV. The epizootic.

- (1) Period before the epizootic.
- (2) Period during the epizootic.
- (3) Period after the epizootic.
- (4) Origin of the epizootic.
- (5) Severity and extent of the epizootic.

#### V. Relation between the epizootic and the epidemic.

- (1) Relation in time.
- (2) Relation in place.

### I. INTRODUCTION.

#### 1. *Situation.*

Dhand is the smaller of the two Punjab villages in which the observations on rat and human plague were carried out. It is situated about eight miles south-west of Amritsar city, with which it has communications by a cart track. The main road from Gharenda to Tarn-Taran passes through the village (*vide* Map 1) dividing it into two parts, a northern part, called the "old village," and a southern portion, the "new village." It is built on practically level ground and covers an area of about 27 acres.

2. *Census results.*

The population according to our census was 1920, and the density of population about 70 per acre. The structure of the houses and of the village generally corresponds to the description already given and it is, therefore, only necessary to add a few census details.

The number of occupied houses is 418. Of these, 121 contain a single room, 105 two rooms and 192 more than two rooms. 110 of the houses are two storeyed, the upper storey usually consisting of a single room. Of the occupied houses only 13 are brick and masonry structures, the remainder being built of sun-dried bricks or clods of caked mud.

The inhabitants may be classified as under:—

Sikhs and Hindus	932
Mahomedans	737
Chuhras	251

It would serve no useful purpose to describe the subdivisions of these groups, but it may be mentioned that the first two groups comprise about twenty distinct castes, most of which have different occupations. While the bulk of the population follows agriculture as a pursuit, the village community includes a large number of persons of the artisan and menial classes, called Kamins, who in return for their services receive a share of the produce of each harvest.

II. PREVIOUS EPIDEMICS OF PLAGUE IN DHAND.

Plague first appeared in Dhand in May, 1902, within two months of its first introduction into the Amritsar District. Six deaths from the disease are recorded to have occurred in May and June of that year. The disease seems to have died out in June and did not reappear till early in November, 1902, when it quickly assumed epidemic proportions. This, which may be taken as the first epidemic, lasted till the middle of March, 1903. There were in all 153 attacks with 81 deaths.

After an interval of nearly 11 months, during which the village remained free from plague, the second epidemic started early in February, 1904, and lasted till the second week of May, 1904. The number of attacks was 376 and of deaths 241.

After another plague-free interval of about 11 months, plague reappeared in April, 1905. The epidemic of that year was a comparatively mild one—47 attacks and 21 deaths. The last recorded death from plague occurred on the 14th July, 1905.

No reliable information could be obtained as to how plague was first introduced into the village in May, 1902, nor as to the probable source of origin of the subsequent epidemics. The result of our inquiries leaves no doubt, however, that during all three epidemics dead rats were found in more or less intimate connection with plague cases.

### III. THE PLAGUE EPIDEMIC OF 1906.

#### 1. *Period before the epidemic.*

We have seen that the last death from plague in 1905 occurred on the 14th July. From the end of November, when the Commission commenced its observations in the village, a careful watch was kept on the health of the people, as has been indicated above. No case of plague came to light until the 6th February, 1906.

#### 2. *Period during the epidemic.*

##### (a) *Severity and duration of epidemic.*

The epidemic of 1906 consisted of 32 cases. The date of attack of the first case was the 6th February and of the last case the 2nd May (Table XIX).

A list of the cases and a short description of each will be found in the Appendix.

##### (b) *Clinical features of the cases.*

1. *Nature of the cases.* Of 27 cases in which a complete examination was made, 25 were of the bubonic form, the other two having no buboes. The remaining five patients were adult females, who would not allow an examination for buboes, but stated that none were present.

2. *Situation of the bubo.* The situation of the bubo in the 25 bubonic cases examined was as follows:—

Femoral .....	15
Axillary .....	5
Cervical .....	3
Femoral and Cervical .....	1
Femoral, Axillary and Cervical ...	1

3. *Mode of onset.* In 16 cases pain in the gland with or without swelling was amongst the earliest symptoms. In three cases the bubo developed on the day following the onset of fever and other symptoms;

in one case after 2 days, in one case after 4 days, in two cases after 5 days and in one case after 6 days.

4. *Sex incidence.* Table XVI shows the relative proportion of males and females which were attacked. It is seen that the incidence on females was considerably higher than that on males.

TABLE XVI.

*Showing the incidence of attacks on males and females in Dhand.*

	Total population	Plague attacks	Incidence per 1000
Males	1037	13	12·5
Females	883	19	21·4

5. *Age incidence.* Table XVII shows the relative incidence on persons at different age periods. While the figures relating to the plague attacks are too small to draw any very definite conclusions, it is seen that the very young and the old are less liable to attack than persons of other ages.

TABLE XVII.

*Showing the relative incidence of attacks on persons at different age periods in Dhand.*

Age period	Total population	Plague attacks	Incidence per 1000
0—5 years	347	1	3
6—10 „	263	9	34
11—20 „	391	8	20
21—40 „	601	11	18
Over 41 „	318	3	9

6. *Caste incidence.* We have already seen that the population can be divided into three very distinct castes. The plague incidence on these three castes is shown in Table XVIII, from which it is seen that the sweepers and menials suffered rather less than the Mahomedans, and the Mahomedans less than the Hindus and Sikhs.

TABLE XVIII.

*Showing the plague incidence on different castes in Dhand.*

Caste	Total population	Plague attacks	Incidence per 1000
Sikhs and Hindus	932	18	19
Mahomedans	737	11	15
Chuhra (menials)	251	3	12

7. *Case mortality.* Out of 32 cases, 19 or almost exactly 60 % ended fatally. The longest interval between the onset of the illness and death was 20 days and the shortest one day; the average for the 19 fatal cases was from four to five days.

(c) *Distribution of cases among houses.*

The 32 cases inhabited 26 houses. Of these one house furnished three cases, four houses furnished two cases each and 21 houses a single case each. Multiple cases in a house were, therefore, uncommon.

All the cases were treated in their own houses for the whole course of their illness. In the 21 houses which furnished single cases the number of contacts was 86, all of whom came into intimate relation with the cases.

(d) *Contact with previous cases.*

We were able definitely to ascertain that 25 (80%) of the cases did not, prior to attack, come in contact with other plague cases (*vide* Appendix), namely: cases *A* to *D*, *F*, *G*, *K* to *S*, *U*, *V*, *X* and *Z* to *FF*. The remaining seven cases, *E*, *H*, *I*, *J*, *T*, *W*, and *Y*, had come in contact with preceding plague cases, so we cannot exclude the possibility of their having been directly infected from the latter. The great majority of cases, however, cannot have received their infection from a previous case.

#### IV. THE EPIZOOTIC.

1. *Period before the epizootic.* (Map 2.) (From 4th December, 1905, to 26th January, 1906.)

During this period 1481 *Mus rattus* were examined at the laboratory, of which 1474 had been trapped and seven were found dead. While none of these rats were shown to be suffering from acute plague, four live rats were found to have chronic abscesses, from which *B. pestis* was recovered. The first of these chronic plague rats was trapped on the 18th December and the last on the 11th January, *vide* Map 2.

As the subject of chronic rat plague has been discussed in a separate paper (Vol. VII. p. 457) we need only point out here that in all four rats *B. pestis* was apparently localised in the abscesses and that there was no evidence that it was present in any of the organs or in the heart-blood.

2. *Period of epizootic.* (Map 1 and Maps 3—16.) (From 27th January, 1906, to 21st April, 1906.)

During this period 542 rats were examined of which number 514 were caught alive and 28 were found dead. Plague was identified in 12 live rats and in 22 dead rats.

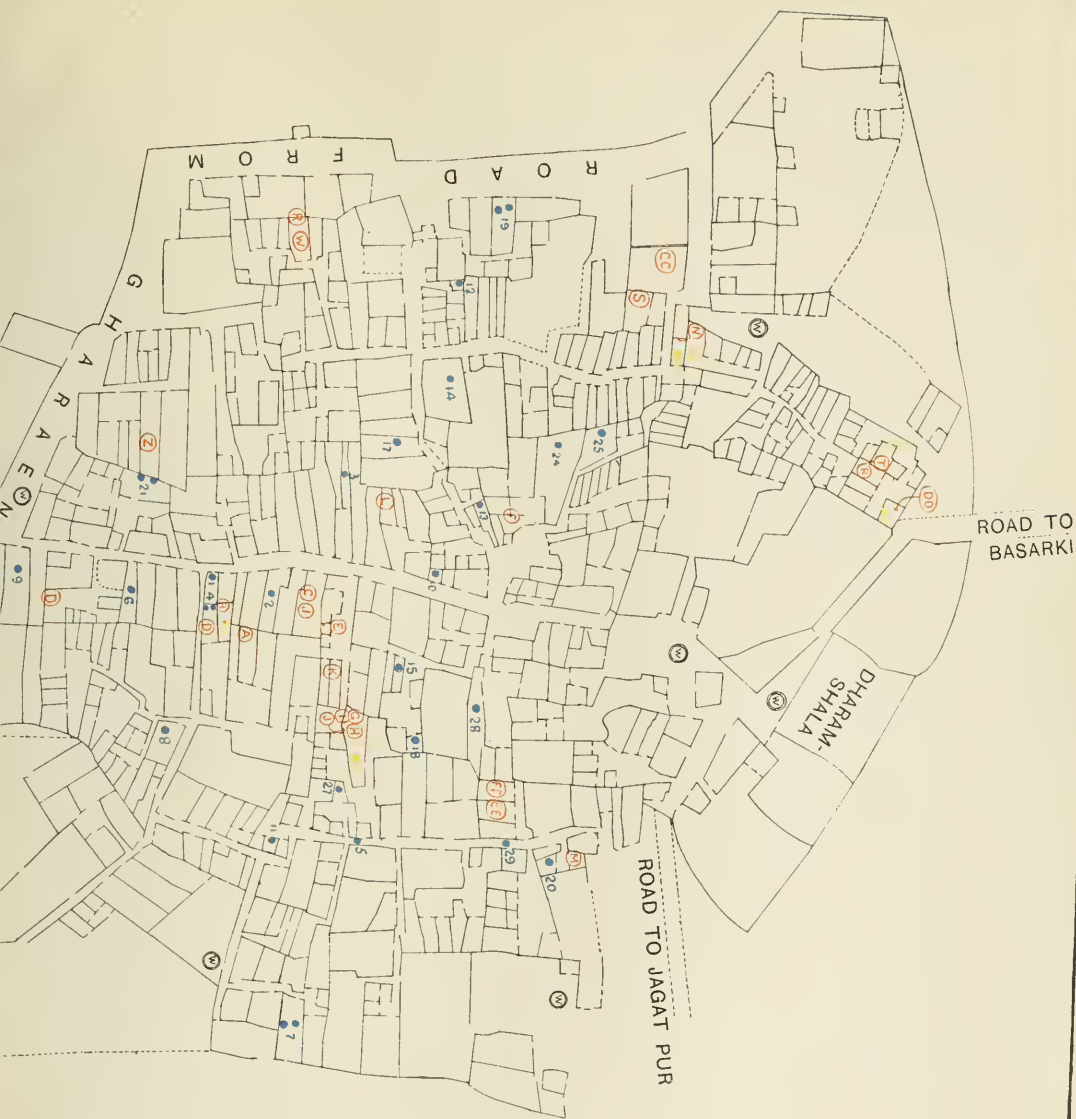


MAP 1

DHAND VILLAGE,  
PUNJAB

Showing all the plague infections





DHARAMSHALA



MAP 2

DHAND VILLAGE,  
PUNJAB

29 Nov. 1906 to 26 Jan. 1906



MAP 2





## DHAND VILLAGE, PUNJAB

Period before the epizootic, 29 Nov. 1905 to 26 Jan. 1906

Scale 100 feet to half-an-inch

● Plague infected rats, all chronic (date)



MAP 3

DHAND VILLAGE,  
PUNJAB

27 Jan. 1906 to 2 Feb. 1906

# MAP 3







## DHAND VILLAGE, PUNJAB

First week of epizootic, 27 Jan. 1906 to 2 Feb. 1906

Scale 100 feet to half-an-inch

● Plague infected rat (date)



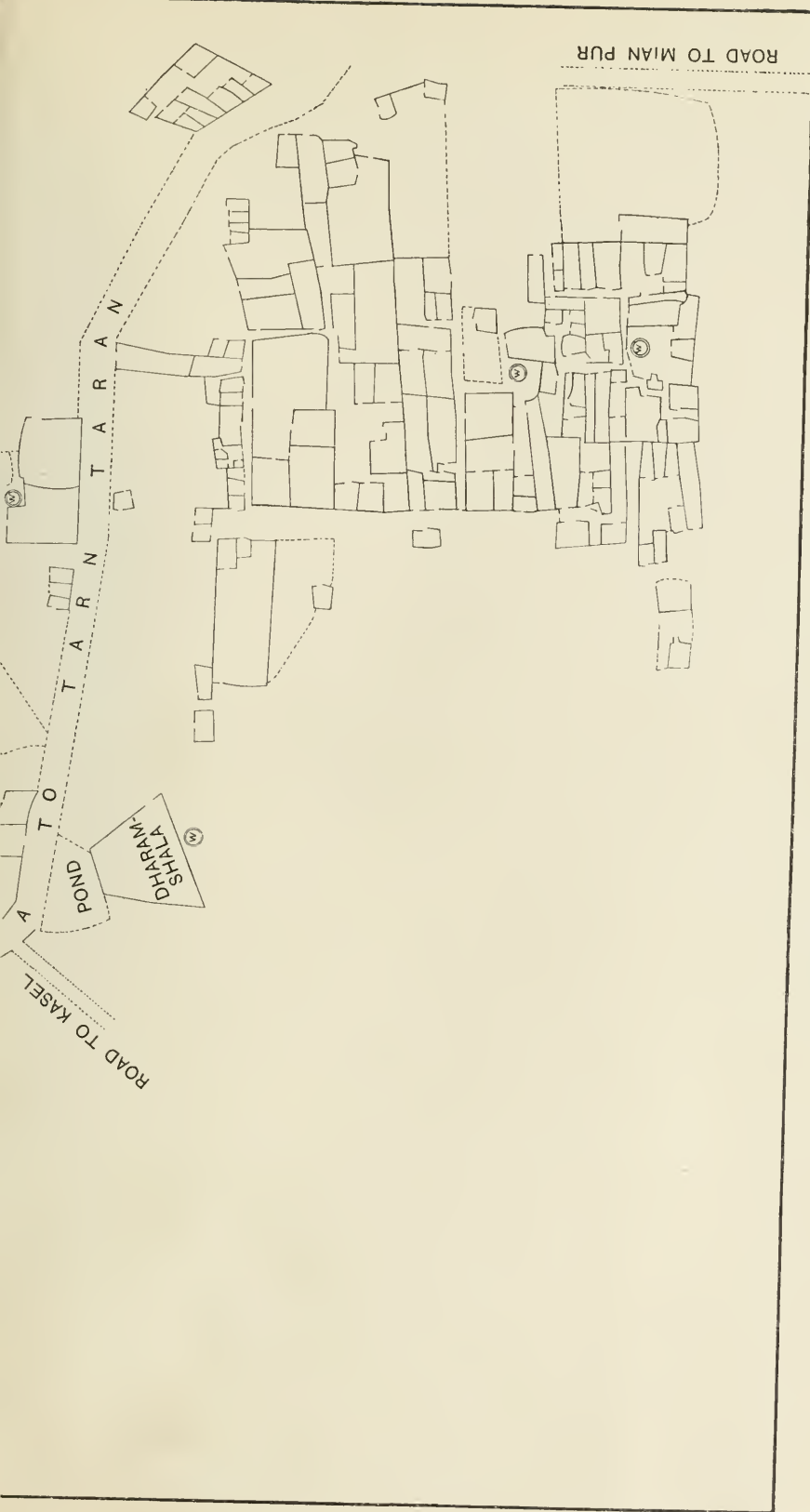
MAP 4

DHAND VILLAGE,  
PUNJAB

3 Feb. 1906 to 9 Feb. 1906 .

# MAP 4





## DHAND VILLAGE, PUNJAB

Second week of epizootic, 3 Feb. 1906 to 9 Feb. 1906

Scale 100 feet to half-an-inch

- Human plague case (date of attack)
- Plague infected rat (date)





MAP 5

DHAND VILLAGE,  
PUNJAB

10 Feb. 1906 to 16 Feb. 1906

# MAP 5



DHARAMSHALA



## DHAND VILLAGE, PUNJAB

Third week of epizootic, 10 Feb. 1906 to 16 Feb. 1906

Scale 100 feet to half-an-inch

- Human plague case (date of attack)
- Plague infected rat (date)



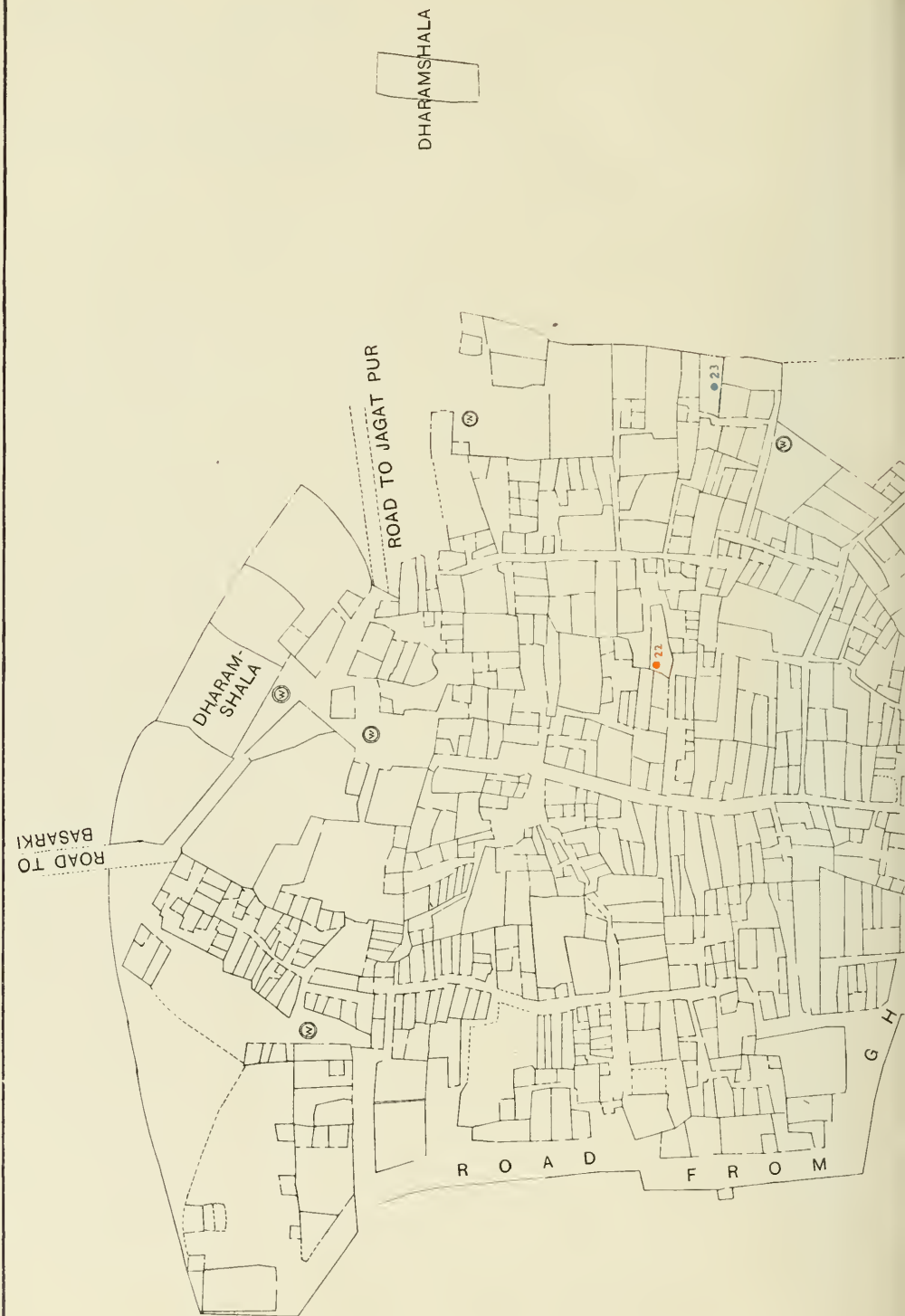


MAP 6

DHAND VILLAGE,  
PUNJAB

17 Feb. 1906 to 23 Feb. 1906

# MAP 6





## DHAND VILLAGE, PUNJAB

Fourth week of epizootic, 17 Feb. 1906 to 23 Feb. 1906



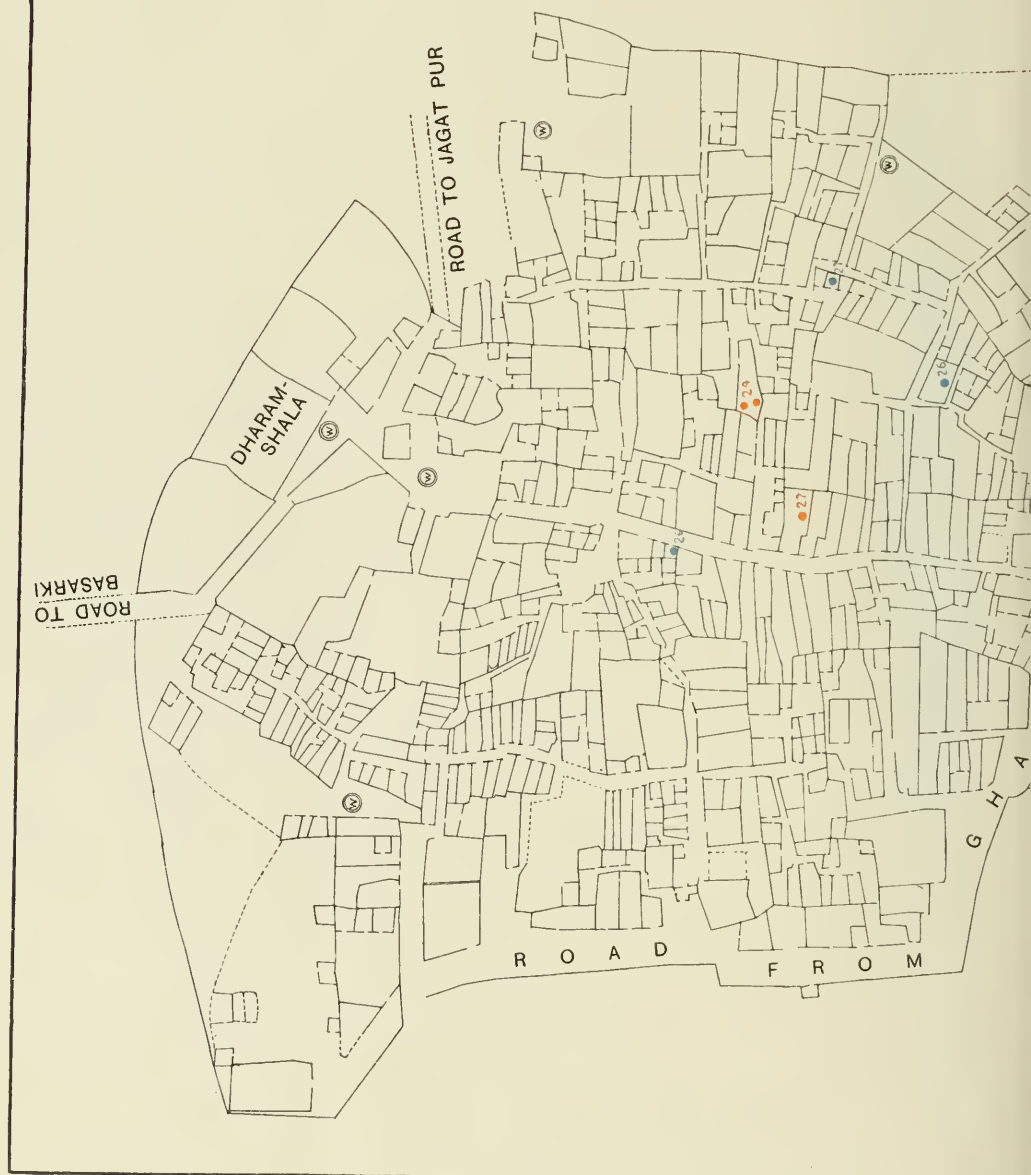
MAP 7

DHAND VILLAGE,  
PUNJAB

24 Feb. 1906 to 2 March, 1906



MAP 7





## DHAND VILLAGE, PUNJAB

Fifth week of epizootic, 24 Feb. 1906 to 2 March, 1906

Scale 100 feet to half-an-inch

● Human plague case (date of attack)

● Plague infected rat (date)

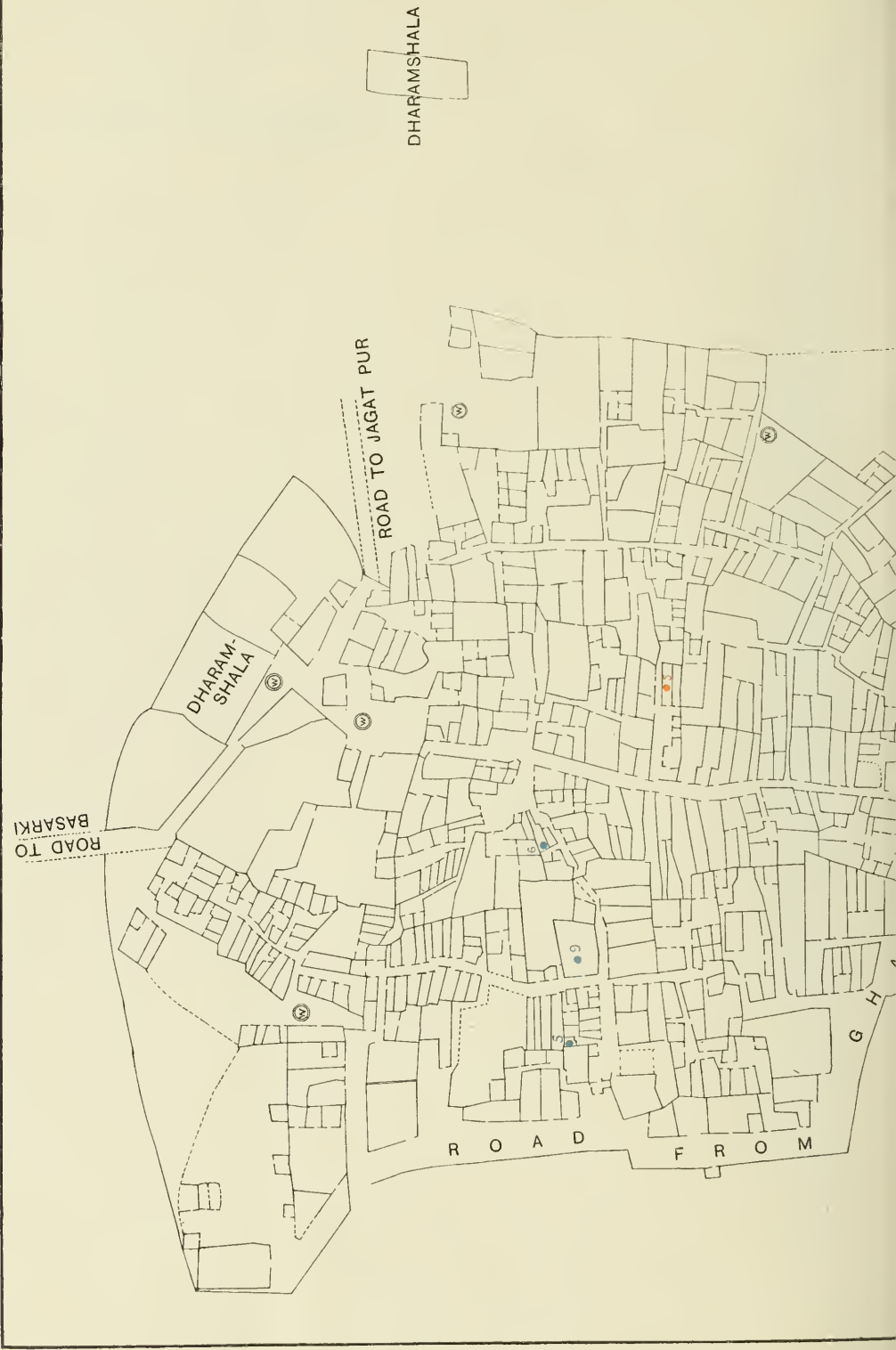


MAP 8

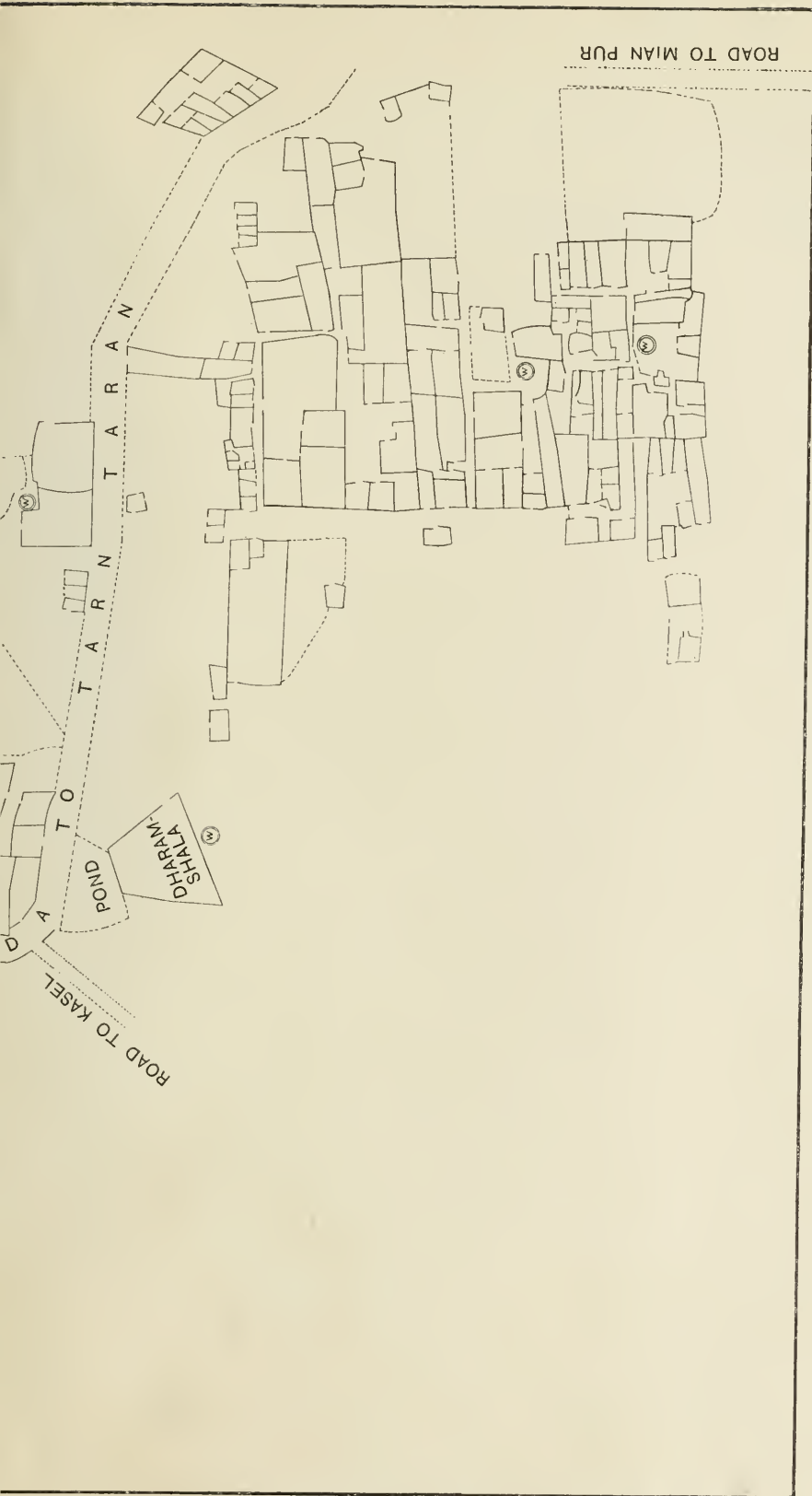
DHAND VILLAGE,  
PUNJAB

3 March, 1906 to 9 March, 1906

MAP 8







## DHAND VILLAGE, PUNJAB

Sixth week of epizootic, 3 March, 1906 to 9 March, 1906

Scale 100 feet to half-an-inch

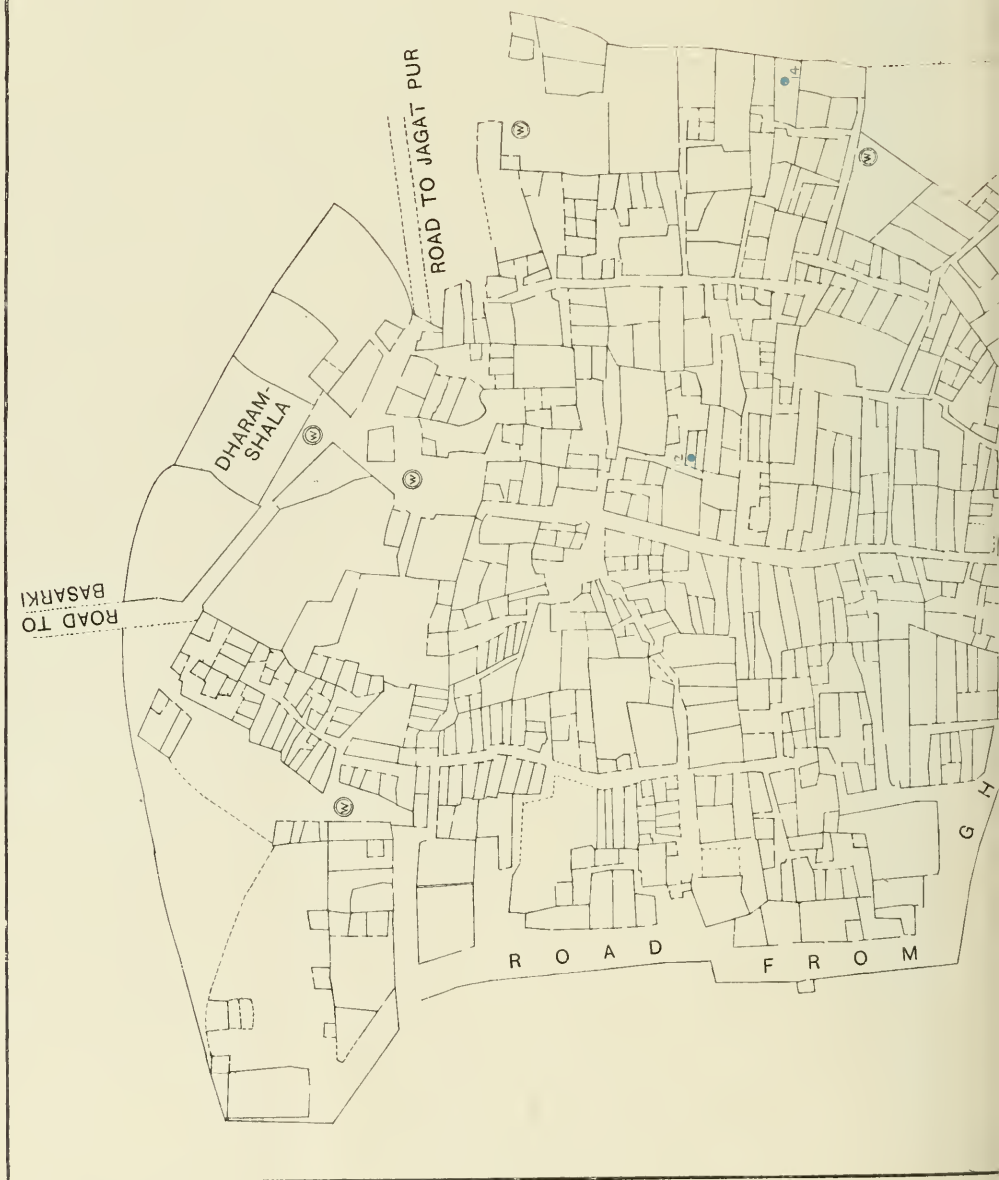


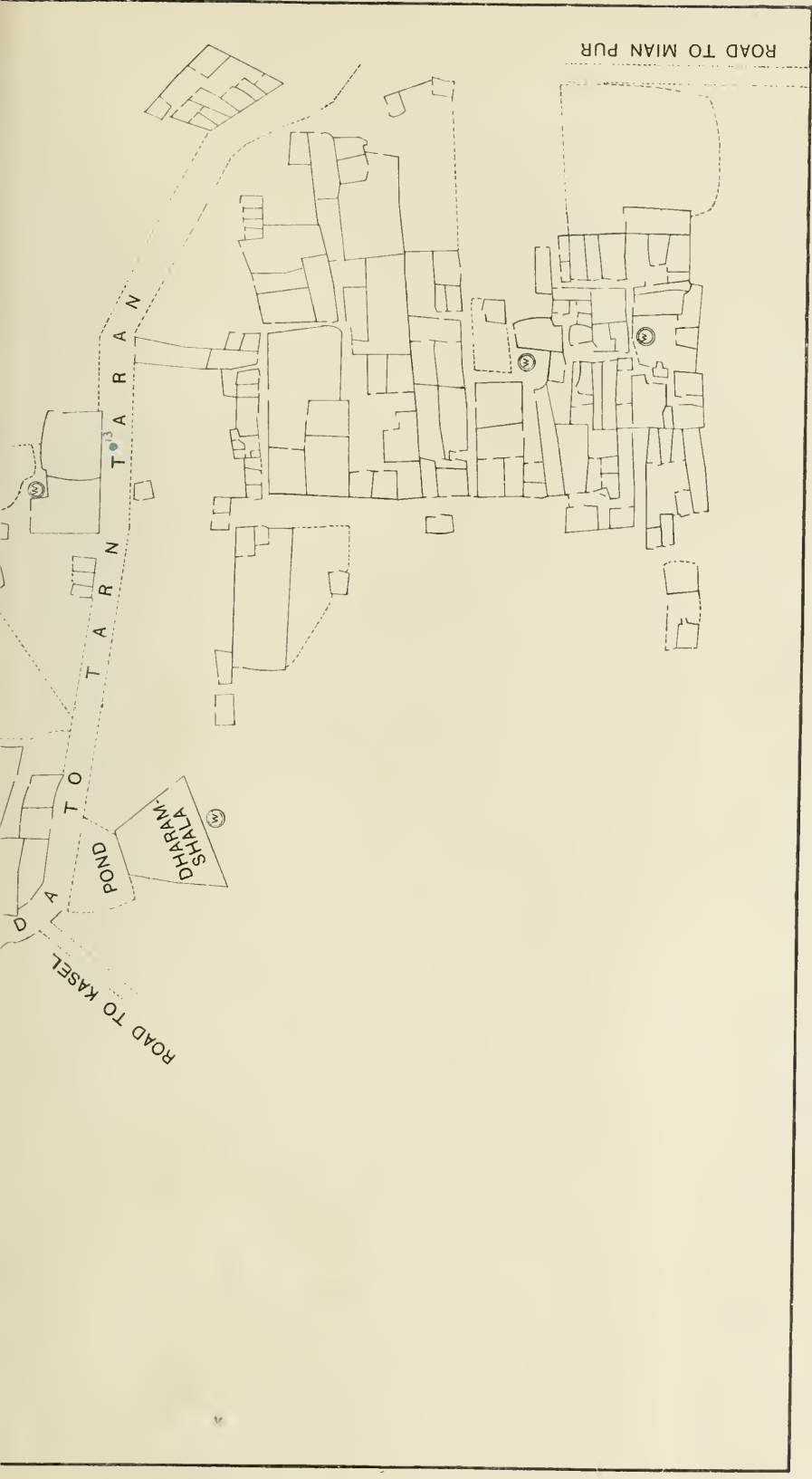
MAP 9

DHAND VILLAGE,  
PUNJAB

10 March, 1906 to 16 March, 1906

MAP 9





# DHAND VILLAGE, PUNJAB

Seventh week of epizootic, 10 March, 1906 to 16 March, 1906

Scale 100 feet to half-an-inch

● Plague infected rat (date)



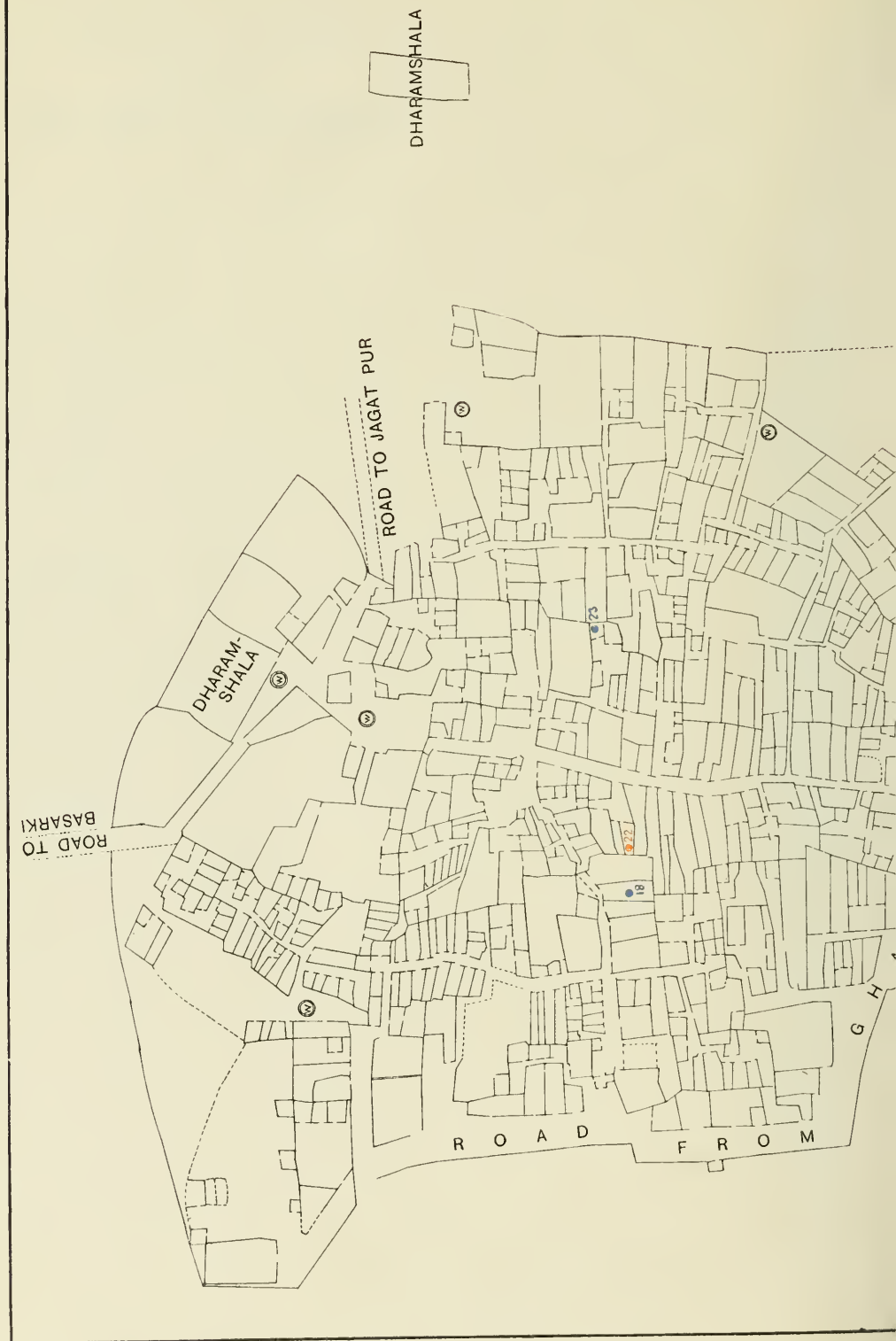


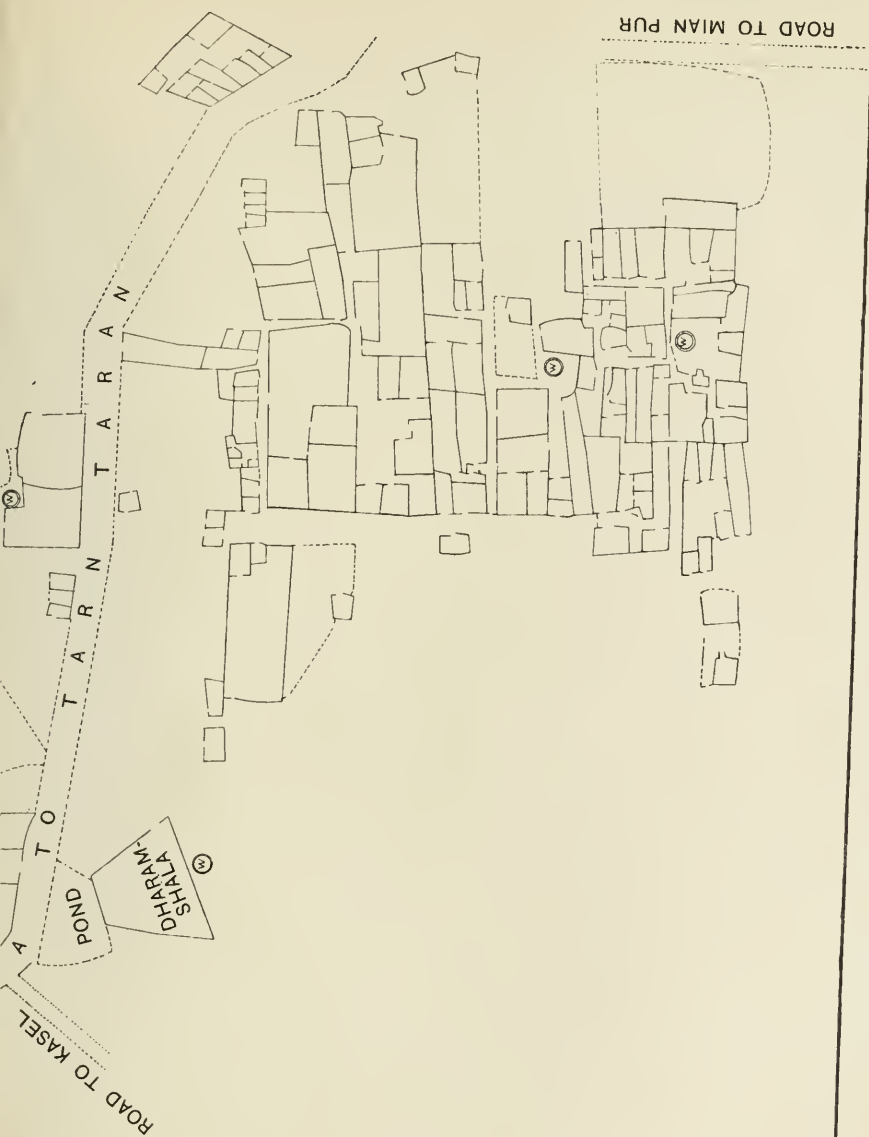
MAP 10

DHAND VILLAGE,  
PUNJAB

17 March, 1906 to 23 March, 1906

MAP 10





## DHAND VILLAGE, PUNJAB

Eighth week of epizootic, 17 March, 1906 to 23 March, 1906

Scale 100 feet to half-an-inch

- Human plague case (date of attack)
- Plague infected rat (date)



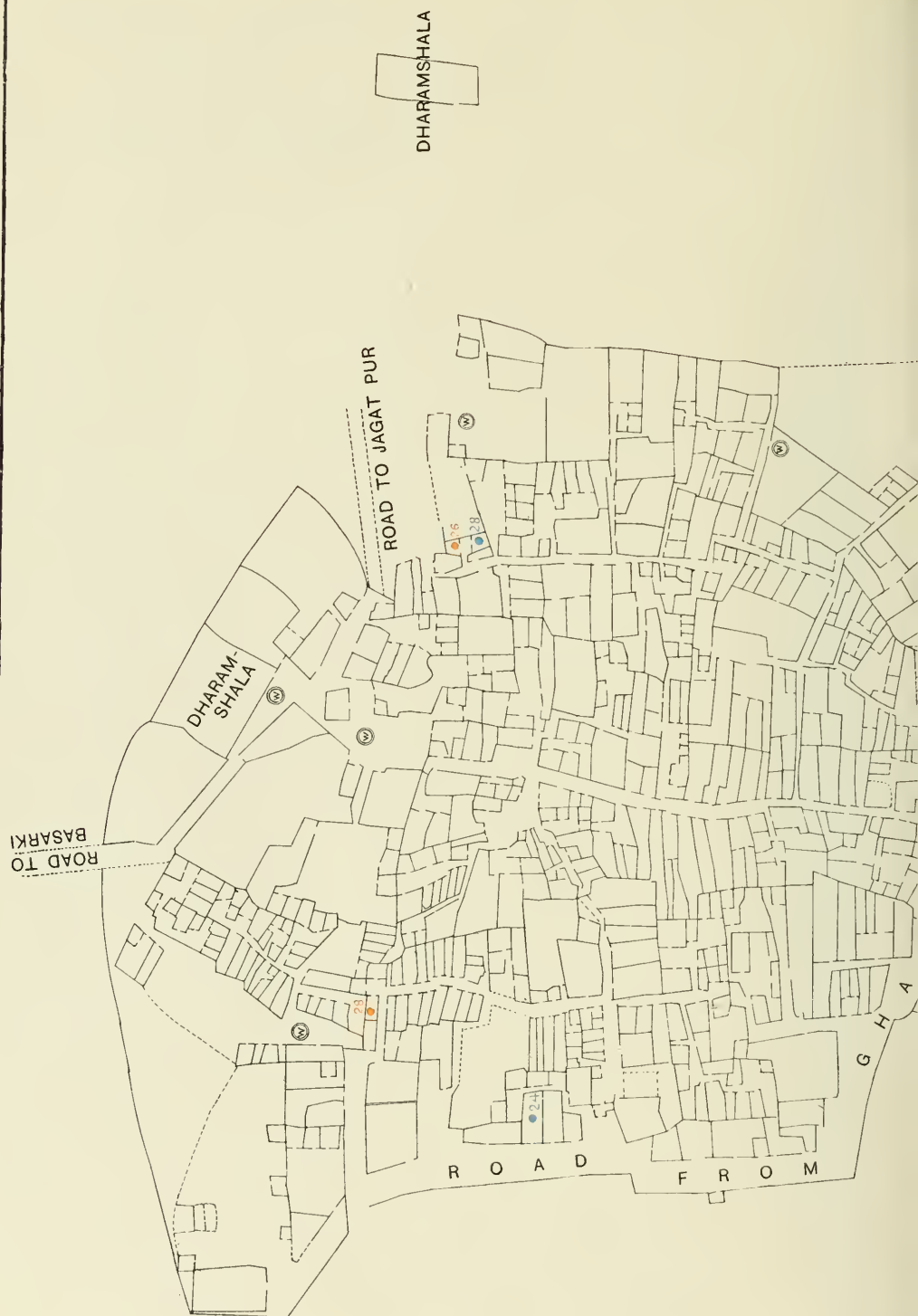


MAP 11

DHAND VILLAGE,  
PUNJAB

24 March, 1906 to 30 March, 1906

# MAP 11





## DHAND VILLAGE, PUNJAB

Ninth week of epizootic, 24 March, 1906 to 30 March, 1906

Scale 100 feet to half-an-inch

- Human plague case (date of attack)
- Plague infected rat (date)



MAP 12

DHAND VILLAGE,  
PUNJAB

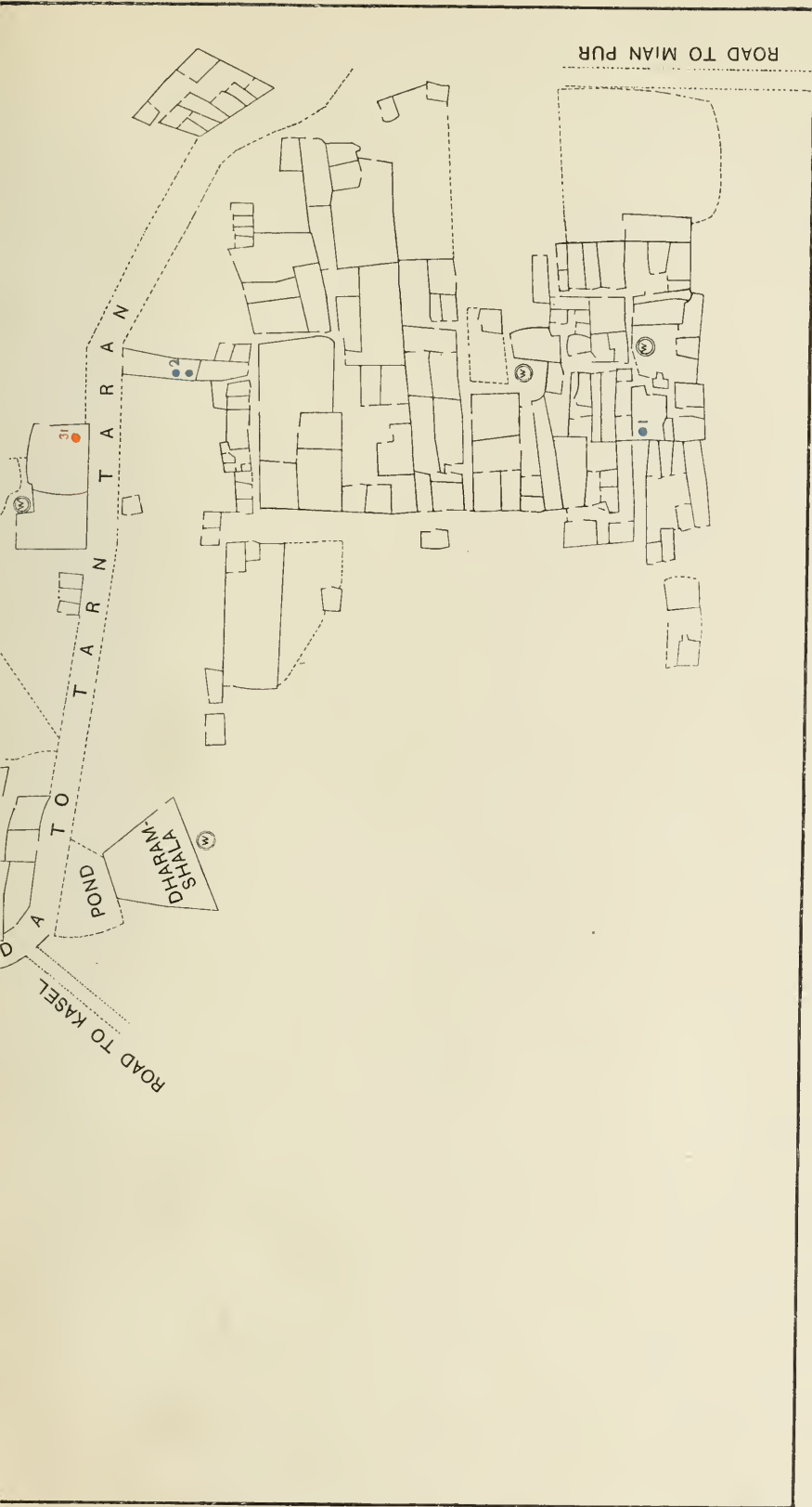
31 March, 1906 to 6 April, 1906



MAP 12



DHARAMSHALA



## DHAND VILLAGE, PUNJAB

Tenth week of epizootic, 31 March, 1906 to 6 April, 1906

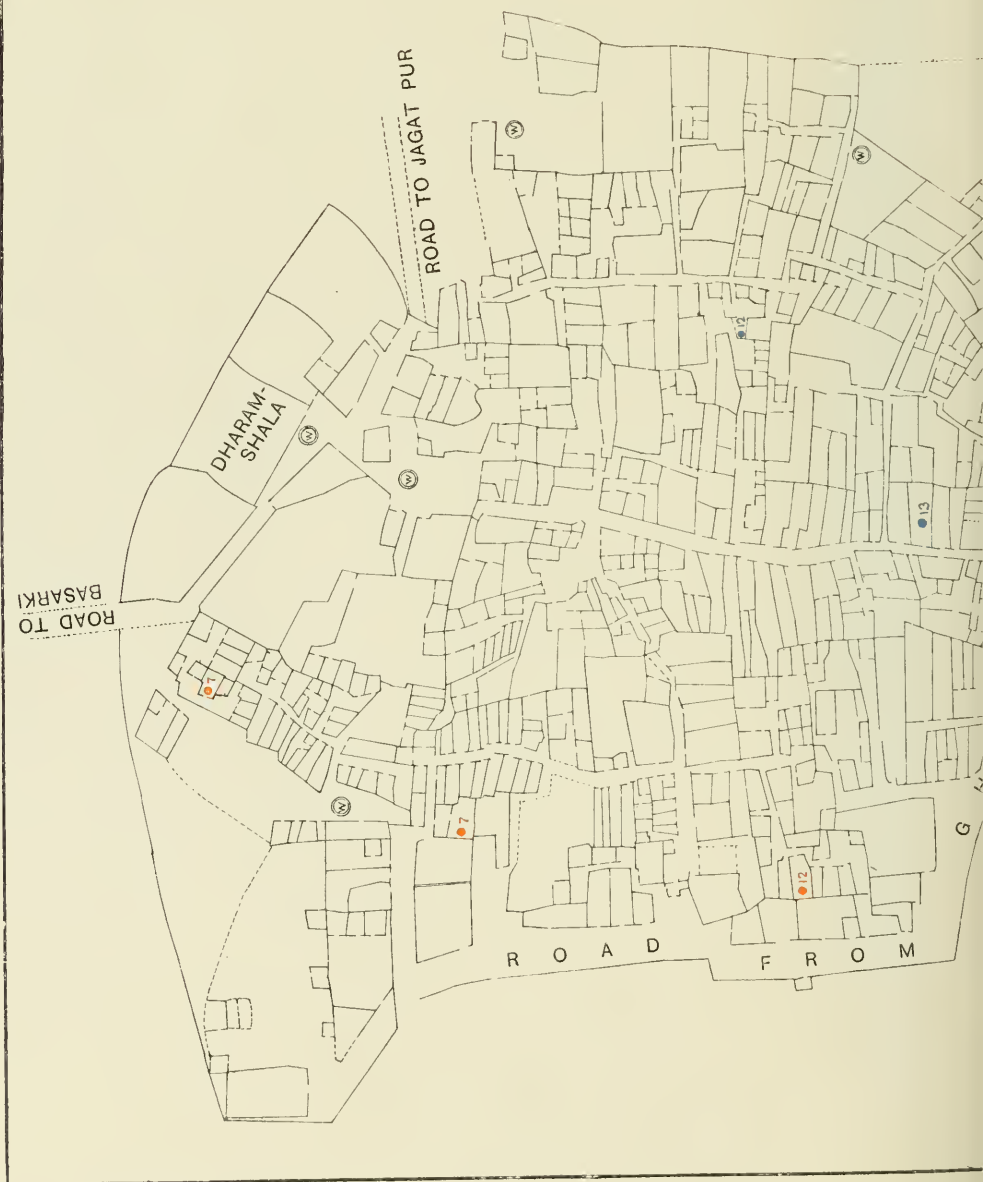


MAP 13

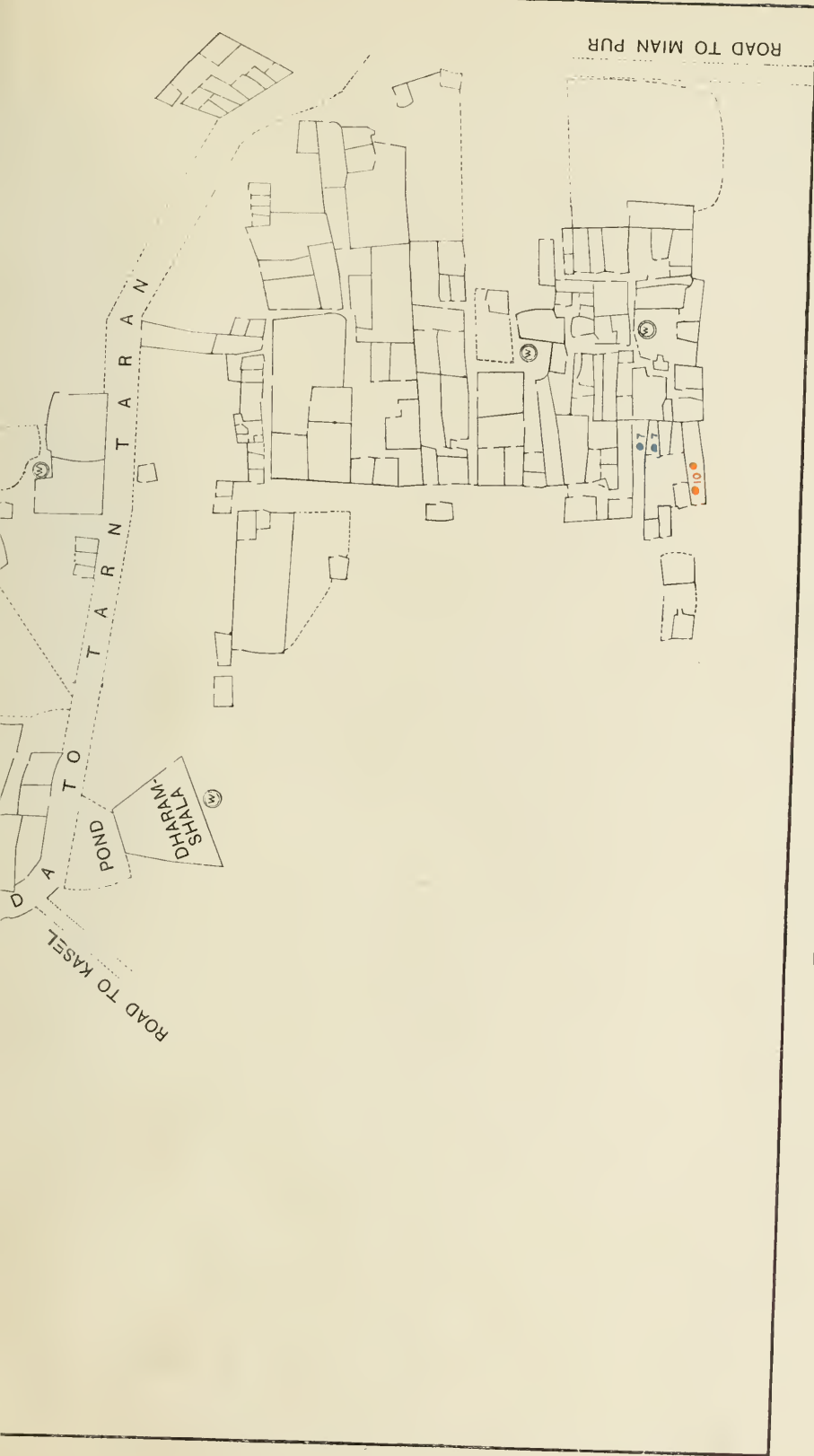
DHAND VILLAGE,  
PUNJAB

7 April, 1906 to 13 April, 1906

# MAP 13







## DHAND VILLAGE, PUNJAB

Eleventh week of epizootic, 7 April, 1906 to 13 April, 1906

Scale 100 feet to half-an-inch

● Human plague case (date of attack)



MAP 14

DHAND VILLAGE,  
PUNJAB

14 April, 1906 to 20 April, 1906

MAP 14





## DHAND VILLAGE, PUNJAB

Twelfth week of epizootic, 14 April, 1906 to 20 April, 1906

Scale 100 feet to half-an-inch

● Human plague case (date of attack)



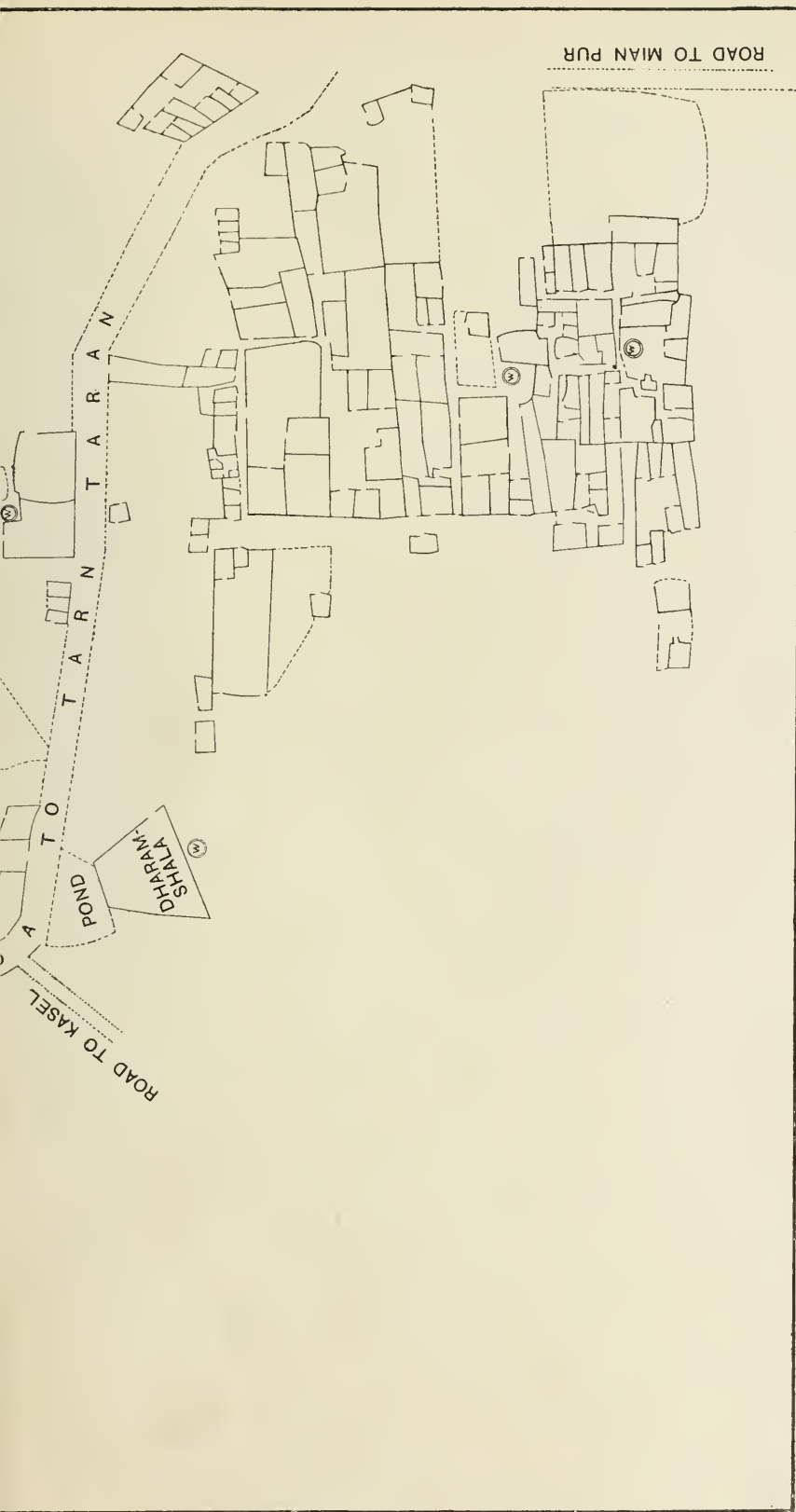


MAP 15

DHAND VILLAGE,  
PUNJAB

21 April, 1906 to 27 April, 1906





## DHAND VILLAGE, PUNJAB

Thirteenth week of epizootic, 21 April, 1906 to 27 April, 1906





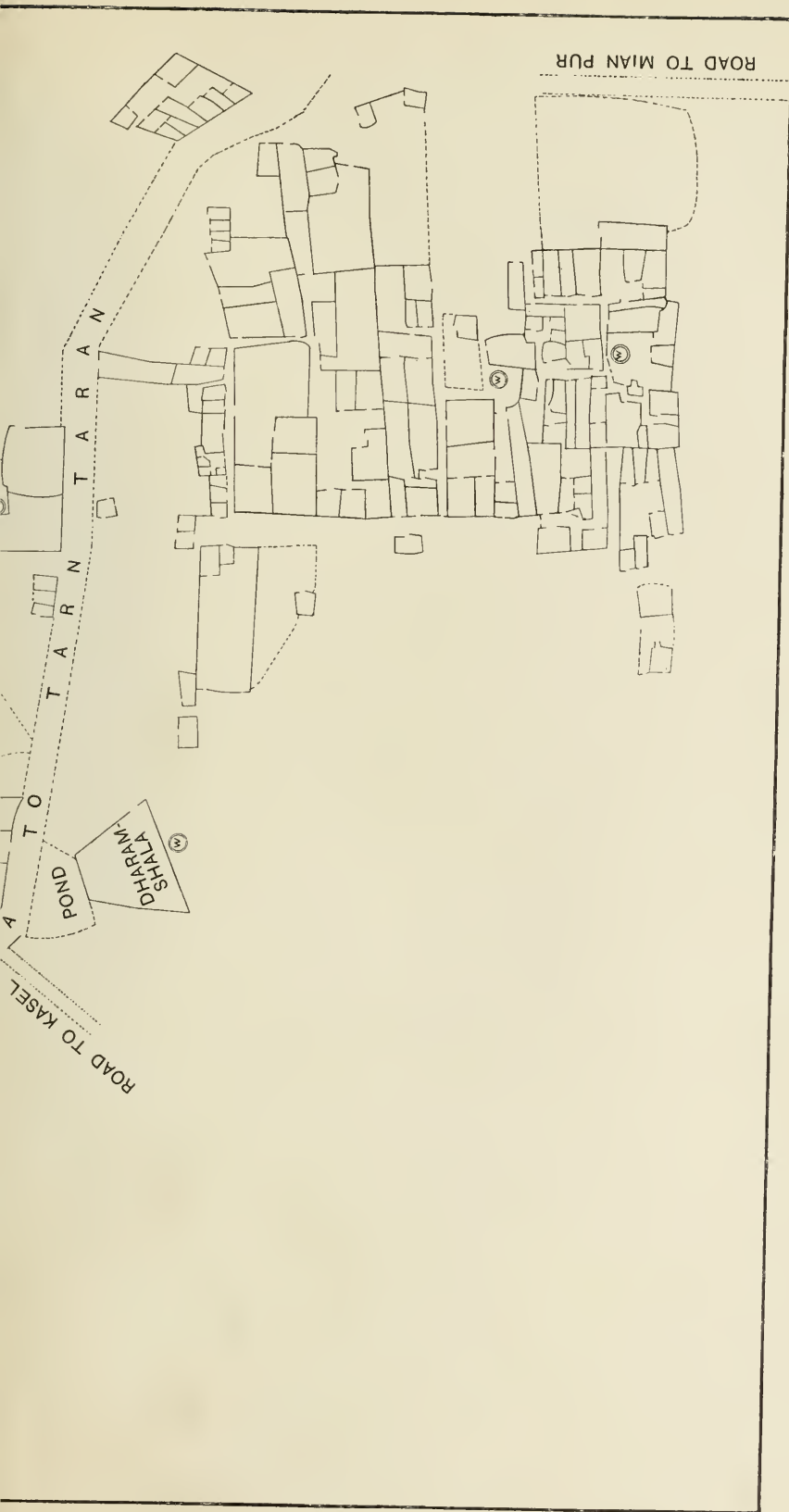
MAP 16

DHAND VILLAGE,  
PUNJAB

28 April, 1906 to 4 May, 1906

MAP 16





## DHAND VILLAGE, PUNJAB

Fourteenth week of epizootic, 28 April, 1906 to 4 May, 1906

Scale 100 feet to half-an-inch

● Human plague case (date of attack)



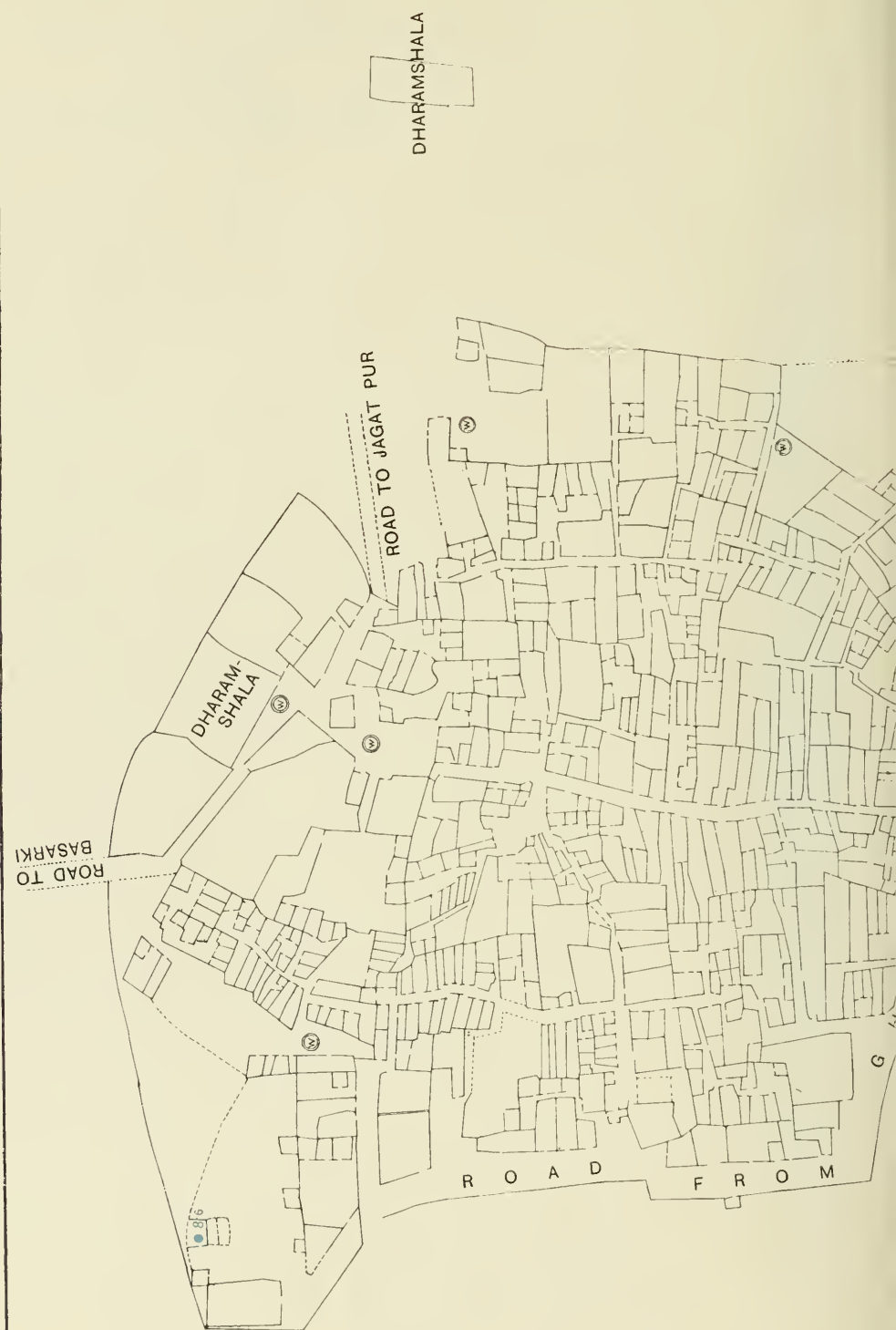
MAP 17

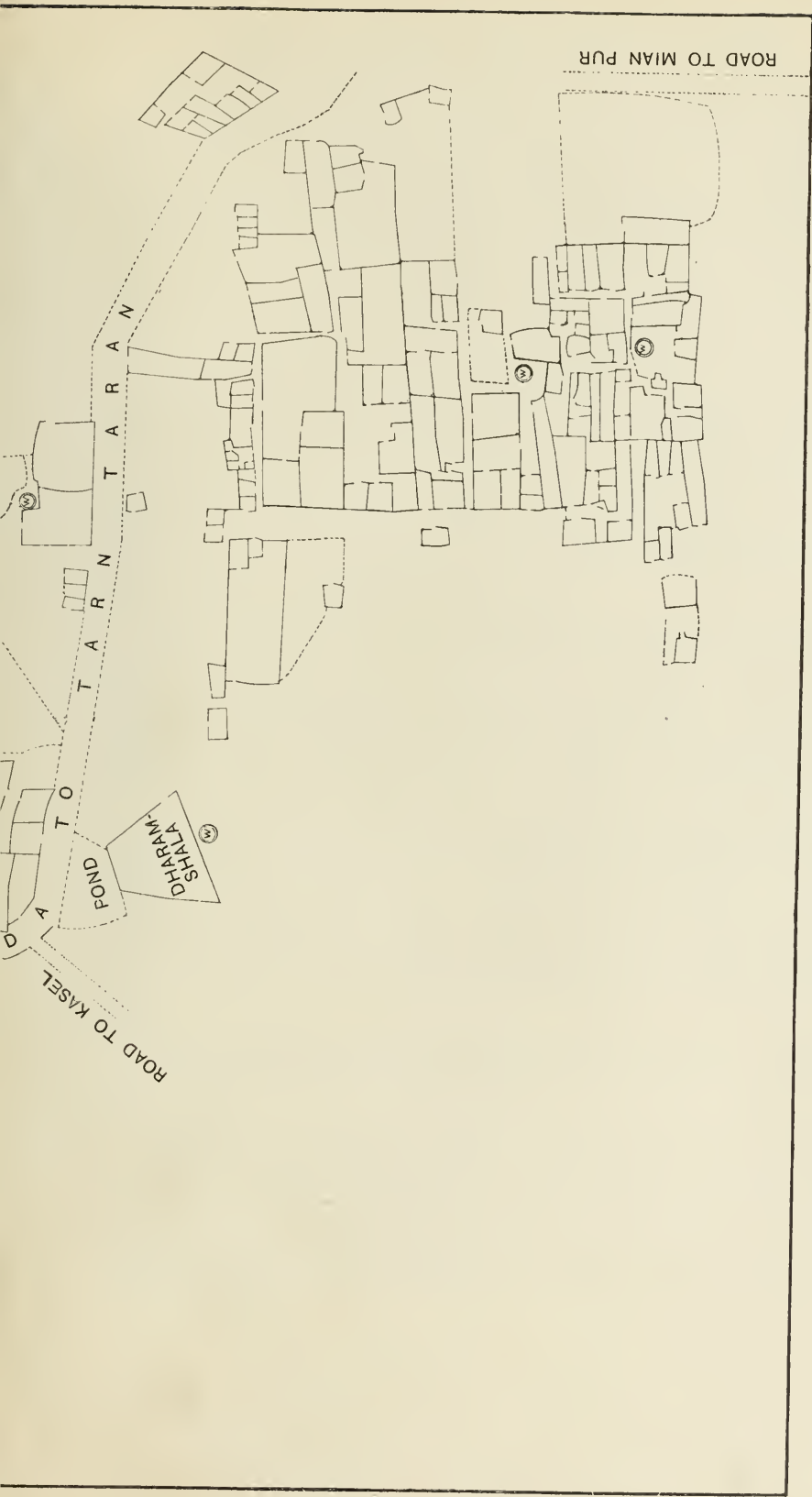
DHAND VILLAGE,  
PUNJAB

5 May, 1906 to 3 December, 1906



MAP 17

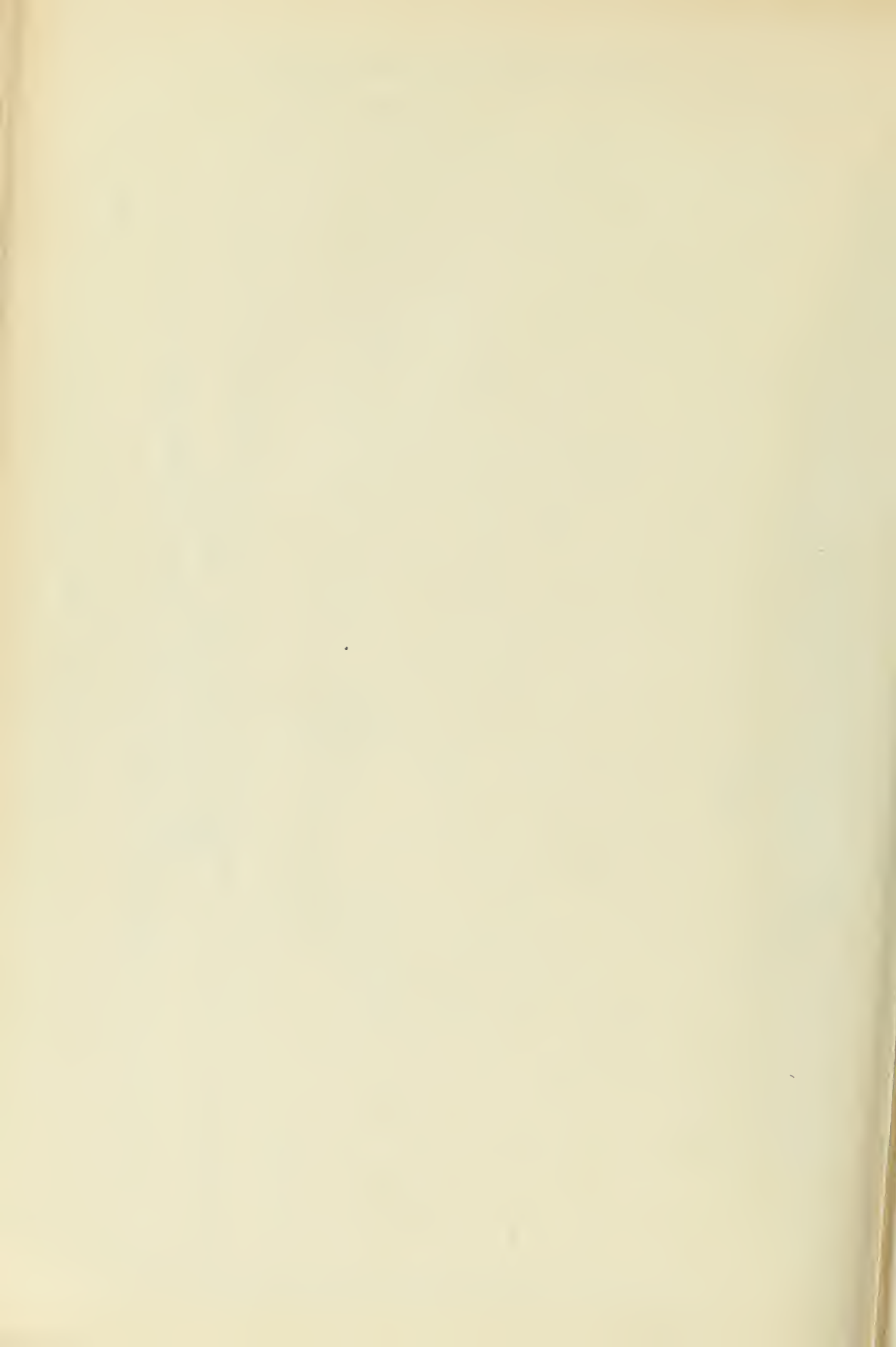




## DHAND VILLAGE, PUNJAB

Period after the epizootic, 5 May, 1906 to 3 December, 1906

● Plague infected rat, chronic (date)



The first acute plague rat was trapped in house No. 347 on the 27th January, *vide* Map 1, Serial No. 1, and Table XIX. This rat had no bubo or other post-mortem lesion but a smear from the spleen showed abundant *B. pestis*. Guinea-pig experiments done on the 29th January to determine if the house was infective gave negative results. On searching the house four mummified carcasses of rats were found in a back room which had remained closed for over a year. This room showed other evidence of rat infestation, the floor being littered with rats' dung and the walls riddled with rats' burrows, apparently leading into an adjoining house No. 350.

The next three plague-infected rats were also trapped alive (Map 1, Serial Nos. 2, 3 and 4), on 30th January and 2nd February. The post-mortem appearances of all three suggested a subacute variety of plague and that the rats were recovering from the acute disease. One had a submaxillary purulent bubo which showed *B. pestis* in the pus. Of the other two, one had a granular liver and the other a granular spleen, but no *B. pestis* were seen in smears from the organs or heart-blood. In all three plague was identified by animal tests.

After an interval of a week two dead plague rats were found on the 9th February (Map 1, Serial Nos. 4 and 5). The former was found in a back room of house No. 350, which immediately adjoins house No. 347, where the first plague rat was trapped, and the latter in a lane some distance away. On the same date in another house adjoining 347, namely, No. 349, four mummified rats were found in a back room.

It is remarkable that, with the exception of the mummified rats referred to (which may or may not have been plague infected), not a single dead plague-infected rat was found until a fortnight after the first acute plague rat had been trapped. In this interval two persons had been attacked with plague, so that if we had depended for our information on the examination of dead rats only, we would have been led to a wrong conclusion as to the time relation of the epizootic and epidemic.

The further progress of the epizootic in Dhand can best be followed by referring to Map 1 and Table XIX, and the series of weekly Maps Nos. 3—16.

The mode of spread of the epizootic through the village was characterised by considerable irregularity. It cannot be said to have extended outwards from the original focus as a wave with a definite margin, leaving the area passed over free from infection for the rest of the epizootic period. On the contrary some plague rats were found at a

TABLE XIX.

*Showing the places at which, and the first and last dates on which, plague-infected rats were taken. The entries are consecutively numbered in accordance with the dates on which the first infected rat was taken. References to the 32 human cases are inserted in the series in their chronological order.*

No.	House No.	Infected rats taken		
		First	Last	Total
1	347	27/1/06	—	1
2	342	30/1/06	—	1
3	156	„	—	1
4	350	2/2/06	9/2/06	2
Case A attacked on 6/2/06 in House No. 352				
„ B	„	7/2/06	„	349
5	In lane near No. 377	9/2/06	—	1
Case C attacked on 12/2/06 in House No. 340				
Cases D and E attacked on 13/2/06 in House Nos. 498 and 338				
6	492	14/2/06	—	1
Case F attacked on 16/2/06 in House No. 181				
„ G	„	22/2/06	„	378
7	439	23/2/06	14/3/06	2
Cases H and I attacked on 24/2/06 in House No. 378				
8	466	26/2/06	—	1
9	501	„	—	1
10	163	„	—	1
11	364	27/2/06	—	1
Case J attacked on 27/2/06 in House No. 340				
12	24	5/3/06	—	1
Case K attacked on 5/3/06 in House No. 335				
13	178	6/3/06	—	1
14	57	9/3/06	—	1
15	321	12/3/06	—	1
16	Near No. 456	13/3/06	—	1
17	60	18/3/06	—	1
Case L attacked on 22/3/06 in House No. 159				
18	318	23/3/06	—	1
19	15	24/3/06	31/3/06	2
Case M attacked on 26/3/06 in House No. 401				
20	402	28/3/06	—	1
Case N attacked on 28/3/06 in House No. 39				
„ O	„	29/3/06	„	603
1	137	31/3/06	3/4/06	2



Case P attacked on 31/3/06 in House No. 456

No.	House No.	Infected rats taken		
		First	Last	Total
22	583	1/4/06	—	1
23	517	2/4/06	—	2
24	185	4/4/06	—	1

Case Q attacked on 4/4/06 in House No. 225

,, R imported ill with Plague on 6/4/06 to House No. 85

25	186	6/4/06	—	1
26	618	7/4/06	—	1

Cases S and T attacked on 7/4/06 in Houses Nos. 3 and 225

,, U and V ,, 10/4/06 in House No. 622

27	371	12/4/06	—	1
----	-----	---------	---	---

Case W attacked on 12/4/06 in House No. 85

Cases X and Y attacked on 14/4/06 in Houses Nos. 587 and 589

Case Z attacked on 18/4/06 in House No. 122

	,, AA	,, 19/4/06	,, ,,	583
	,, BB	,, 20/4/06	,, ,,	588
28	315	21/4/06	—	1
29	Near 408	,,	—	1

Case CC attacked on 21/4/06 in House No. 2

,, DD	,, 23/4/06	,, ,,	220
,, EE	,, 1/5/06	,, ,,	386
,, FF	,, 2/5/06	,, ,,	387

considerable distance from the original focus of infection early in the epizootic, such as Nos. 7 and 12; while many of the later plague rats were found quite near the area where the epizootic had begun, pointing either to the persistence of infection in this area or to re-infection from more peripheral areas, *vide* Serial Nos. 15, 18, 21, 27, and 28. In spite, however, of these irregularities it may be admitted that the general direction of spread was centrifugal, and that the areas furthest from the original focus were the last to become infected. An instance of this is furnished by the southern part of the village, in which the first infected rat, Serial No. 22, was found on 1st April, more than two months after the origin of the epizootic.

Similarly the first evidence of the spread of the epizootic to the sweepers' quarters in the extreme north of the village was obtained by the finding, early in April, of infected rat fleas in house No. 225 in which case Q occurred.

### 3. *Period after the epizootic.* (Map No. 17.)

The last acute plague rats were taken on the 21st April 1906. From the 22nd April to the conclusion of the observations in Dhand on the 3rd December 1906, 592 rats were examined, of which 583 were trapped alive and nine were brought dead to the laboratory. On the 8th June a plague-infected live rat was taken (*vide* Map 17). This rat had a purulent submaxillary bubo and no other lesions. Smear preparations of the pus showed several clumps of plague-like organisms. A guinea-pig inoculated cutaneously with the pus died of plague in four days. No other plague-infected rat was found from this date to the conclusion of the observations.

### 4. *Origin of the epizootic.*

We were not able to obtain any evidence which would enable us to come to a conclusion as to the origin of the epizootic in Dhand.

It is practically certain that no person suffering from or incubating plague arrived in the village before the epizootic began. As regards the question of the introduction of plague by healthy persons, who had been exposed to infection elsewhere, we may say that although we made careful inquiries in all the houses in the neighbourhood of which the epizootic began, we failed to elicit any information pointing to the disease having been introduced in this way. It is true that at this time many of the villagers used to go to and fro between Dhand and Amritsar, which was infected, and it is impossible to exclude this mode of origin, more especially as a certain class of the villagers are not disposed to admit that they have visited friends or relatives ill with plague in other villages or towns.

On the other hand, we may exclude the possibility of introduction by migration of infected rats, as at this time, so far as could be ascertained, there was no infected village or town within five miles of Dhand. In this connection it is to be noted that a chronic plague rat was found on the 11th January, or only about a fortnight before the first acute plague rat was taken. We have, however, fully discussed elsewhere (vol. VII. p. 457) the question of the lighting up of chronic into acute plague and we need not enter into it here.

We must then leave the question of the origin of the epizootic unsettled.

5. *Severity and extent of the epizootic.*

The epizootic in Dhand was apparently of very moderate severity. The number of plague-infected rats found was only 34 as compared with 261 in Kasel. If we add to the latter figure 89 dead rats found in Kasel during the epizootic period, which were too putrid for diagnosis, the plague mortality among the rats in Kasel becomes ten times as great as in Dhand.

We must, however, point out that we have reasons for supposing that in Dhand a larger proportion of the dead rats found were concealed than in Kasel. But, after making full allowance for this possibility and for the difference in the size of the villages, there is no question that the Dhand epizootic fell far short of the Kasel epizootic in point of severity.

We think that this difference was probably mainly due to the relatively sparse rat population in Dhand at the time the epizootic broke out. A comparison of the number of rats taken per 100 traps set in the two villages during their respective epizootic periods (Tables Nos. IX and X) shows that the rat infestation of Dhand was relatively extremely low. The great diminution of the rat population in Dhand seems also to afford the best explanation of the termination of the epizootic at a time when fleas were prevalent and the temperature was favourable for the spread of the disease.

Apart from the limitation of the epizootic in Dhand, which was indicated by the small number of plague-infected rats found, it is interesting to note that the disease appeared to be of a less virulent type here than in Kasel. Thus, the number of live plague rats, most of which had the disease in a subacute form or appeared to be recovering from an acute attack, was 44% of the total number of plague rats as compared with 13% in Kasel.

V. RELATION BETWEEN THE EPIZOOTIC AND EPIDEMIC.

The relation between the epizootic and epidemic can best be seen by a study of the maps appended and of Tables XIX and XX.

(1) *Relation in time.*

The relation in time becomes apparent by reference to Tables XIX and XX, which latter shows the number of infected rats and the number of plague attacks and deaths for each week of the period during which observations in Dhand were carried out.

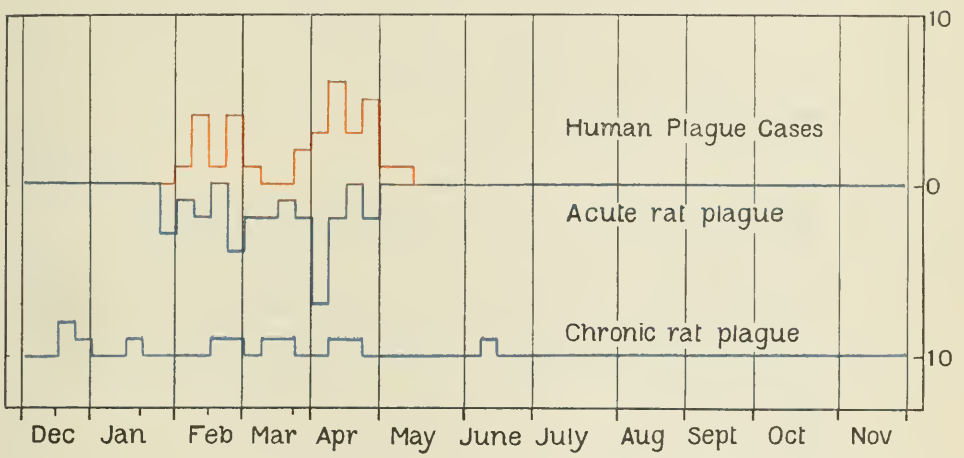
*Plague in Dhand*

TABLE XX.

*Showing number of plague cases and plague-infected rats in  
Dhand week by week.*

Week ending	Plague cases	Plague deaths	Rats			Grand total
			Acute plague	Too putrid for diagnosis	Chronic plague	
5/12/05	—	—	—	—	—	—
12/12/05	—	—	—	—	—	—
19/12/05	—	—	—	—	2	2
26/12/05	—	—	—	—	1	1
2/1/06	—	—	—	—	—	—
9/1/06	—	—	—	—	—	—
16/1/06	—	—	—	—	1	1
23/1/06	—	—	—	—	—	—
30/1/06	—	—	3	—	—	3
6/2/06	1	—	1	—	—	1
13/2/06	4	—	2	—	—	2
20/2/06	1	1	—	—	1	1
27/2/06	4	2	4	—	1	5
6/3/06	1	1	2	—	—	2
13/3/06	—	1	2	—	1	3
20/3/06	—	—	1	—	1	2
27/3/06	2	1	2	—	—	2
3/4/06	3	2	7	—	—	7
10/4/06	6	2	2	—	1	3
17/4/06	3	2	—	1	1	2
24/4/06	5	2	2	—	—	2
1/5/06	1	2	—	—	—	—
8/5/06	1	2	—	—	—	—
15/5/06	—	1	—	—	—	—
22/5/06	—	—	—	—	—	—
29/5/06	—	—	—	—	—	—
5/6/06	—	—	—	—	—	—
12/6/06	—	—	—	—	1	1
19/6/06	—	—	—	—	—	—
26/6/06	—	—	—	—	—	—
3/7/06	—	—	—	—	—	—
10/7/06	—	—	—	—	—	—
17/7/06	—	—	—	—	—	—
24/7/06	—	—	—	—	—	—
31/7/06	—	—	—	—	—	—
7/8/06	—	—	—	—	—	—
14/8/06	—	—	—	—	—	—
21/8/06	—	—	—	—	—	—
28/8/06	—	—	—	—	—	—
4/9/06	—	—	—	—	—	—
11/9/06	—	—	—	—	—	—
18/9/06	—	—	—	—	—	—
25/9/06	—	—	—	—	—	—
2/10/06	—	—	—	—	—	—
9/10/06	—	—	—	—	—	—
16/10/06	—	—	—	—	—	—
23/10/06	—	—	—	—	—	—
30/10/06	—	—	—	—	—	—
6/11/06	—	—	—	—	—	—
13/11/06	—	—	—	—	—	—
20/11/06	—	—	—	—	—	—
27/11/06	—	—	—	—	—	—

91



Human and rat plague in Dhand





It will be seen from Table XIX that the first acute plague rat was taken on the 27th January and the last on the 21st April, while the first human case was attacked on 6th February and the last on 2nd May.

It is at once evident, then, that in Dhand, (1) rat plague preceded human plague, and (2) human plague ceased shortly after the cessation of plague among the rats.

## 2. *Relation in place.*

We propose only briefly to refer to different groups of cases.

*Cases A, B, C and E* lived in the neighbourhood of the place where the first plague-infected rats were found.

*Case D* is shown on Map 1 both at his residence and at the house where he was probably infected. He had removed a dead plague rat from the latter house four days prior to his attack.

*Case F.* Previous to attack no plague rat had been found in the vicinity of the residence of this case. The patient was a child, aged seven, whose movements prior to her illness could not be accurately ascertained.

*Cases G, H and I* lived in house No. 378. *Cases H and I* were attacked within a few hours of each other and two days after case *G* became ill. Thirteen days before case *G* was attacked a plague-infected rat was found in a lane not far from this house. The house was shown to be infective to guinea-pigs on the day following the attack of *G*.

*Case J*, who lived in another house, No. 340, was a frequent visitor to this infected house (No. 378) during the illness of *G, H and I*, being a near relative of the family. It will be seen from the map that previous to *J*'s attack there had been a case (*C*) in the house in which she resided (No. 340). The interval between the attacks, namely, a fortnight, and the fact that the house, No. 340, was not found infective to guinea-pigs three days after case *C*'s attack, suggest that case *J* got infected elsewhere.

*Case K* lived in a house immediately adjoining the infected house, No. 378.

*Cases L and M.* Plague-infected rats were found in the houses adjoining those in which both these cases lived.

*Case N.* No infected rats were found in or near the residence of this case, but guinea-pig experiments showed that the house contained infected rat fleas.

*Case O.* This was the first case that occurred in the "New village." No plague rats had been found in this portion of the village prior to *O*'s

attack, but three days afterwards a live infected rat (No. 22) was taken in a house not far from her residence. It will be seen from the Map (No. 1), that this house is a long way away from the old village, from which it is separated by the greater part of the new village. No plague-infected rats were found at any time in this intervening part of the new village. These facts do not favour the supposition that the epizootic spread by contiguity from the old to the new village. They seem, on the contrary, to suggest that infection, introduced by human agency, probably by case *O*, gave rise to an epizootic of plague among the rats in the neighbourhood. It was ascertained that case *O* used to go to the old village daily to get lessons in sewing, but we could not get any history of her having visited at a house which had been proved to be infected.

*Case P.* A plague-infected rat was found in the immediate vicinity of the residence of this case 18 days prior to his attack.

*Cases Q<sup>1</sup>, T, and DD.* No plague-infected rats were found in or near the residences of these cases. The house which furnished cases *Q* and *T* was, however, proved to contain infected rat fleas, while the house occupied by *DD* was shown to be infective to a guinea-pig.

*Cases R and W.* *Case R* was attacked with plague on 5th April in another village (Chabal) and was brought to Dhand next day. *Case W*, who is a brother of case *R*, had lived with him in Chabal and had come to Dhand with him: he was attacked six days after leaving Chabal. Dead rats were found at the time case *R* became ill in the house in which these cases lived in Chabal. *Case R* was, therefore, undoubtedly an imported case. *Case W* may have been infected in Chabal, in which case his incubation period would have been at least six days: or, again, he may have been infected after his arrival in Dhand by means of fleas which had been brought from his infected house in Chabal. No plague rats were found in the immediate neighbourhood of the house they occupied in Dhand.

*Cases U, V, X, Y, AA and BB* all occurred in the new village, in association with plague among the rats in the neighbourhood.

*Case Z.* Plague rats had been found a fortnight previously in a store for chaff, which immediately adjoined the residence of this case.

*Cases EE and FF* occurred in adjoining houses. Plague-infected rats had been found ten days previously on both sides of their houses.

Analysing the 32 plague cases with reference to their association or

<sup>1</sup> Marked *R* in Map 1, in northernmost part of village.

otherwise with plague rats, we may divide them into the following two groups:—

Group I: comprising 22 cases, inhabiting 18 houses, in which or in the vicinity of which plague rats were found before or at the time of the occurrence of the cases. The cases included in this group are:—*A, B, C, D, E, G, H, I, J, K, L, M, P, U, V, X, Y, Z, AA, BB, EE* and *FF*.

Group II: comprising 10 cases, inhabiting 8 houses, in which or in the vicinity of which no plague rats were found before or at the time the cases were attacked. The cases in this group are *F, N, O, Q, R, S, T, W, CC* and *DD*. Three of these 8 houses, furnishing 4 cases, namely, *N, Q*<sup>1</sup> and *T*, and *DD*, were shown by guinea-pig experiments to contain infective rat fleas (Table XXI).

TABLE XXI.

*Showing houses which furnished plague cases, but not plague rats, and in which the house was shown to be infective by guinea-pig experiments. The experiments are numbered consecutively in chronological order.*

No.	House No.	Date on which guinea-pig was put in the house	Reference to case, and remarks
I	349	9/2/06	Case B: guinea-pig died of plague
II	378	23/2/06	Cases G, H and I: guinea-pig died of plague
III	39	31/3/06	Case N: two guinea-pigs died of plague and fleas from house infected a guinea-pig in the laboratory
IV	225	10/4/06	Cases Q and T: fleas from guinea-pig put down in house infected a guinea-pig in laboratory
V	220	25/4/06	Case DD: guinea-pig died of plague

Of the remaining 6 cases, 2, namely, *R* and *W*, were imported, and 2 others, namely, *S* and *CC*, lived in the neighbourhood of one of the houses shown to contain infected rat fleas. The remaining 2 cases, namely, *F* and *O*, were children whose movements before their illness could not be accurately determined. It may be mentioned, however, that plague rats were found in the vicinity of the houses of cases *F* and *O*, 10 days and 3 days, respectively, after they fell ill.

Summarising these observations on the place relation of the epidemic to the epizootic in Dhand, we may say that, while we were able to show that human plague occurred in association with rat plague in the near neighbourhood, we usually failed to establish a similar association in individual houses in which cases occurred. Our failure to trace this more intimate association between plague cases and plague rats must,

<sup>1</sup> Marked R in Map 1, in northernmost part of village.

we consider, have been largely due to the limitation of our methods of search for the latter, and perhaps to concealment on the part of the villagers who had not quite got accustomed to our presence at the time of the epidemic.

Finally, in attempting to estimate the closeness of the association between human plague and rat plague in a village such as Dhand we must take into account the small size and close aggregation of the houses and the free communication by means of rat burrows between neighbouring houses.

It will then be readily apparent that in Dhand the association which existed between plague cases and plague rats found in an adjoining house or even plague rats found in a house near by, but not immediately adjoining, may often have been more intimate than that which often obtains between human cases and plague rats found in different rooms or on different floors, of large premises, such as exist in cities.

It follows from what we have just said that in Dhand the appearance of plague rats in houses was not often followed by the occurrence of plague among the inmates.

Excluding 12 live plague-infected rats, we found in Dhand during the epizootic 22 dead plague rats. Of these, 14 were found in lanes, unoccupied houses or godowns, and hence not in close association with human beings. The remaining eight plague rats were found in six occupied houses, either in the actual living rooms, or in rooms directly communicating with them. These six houses contained 31 inmates. Two houses, containing seven occupants, were evacuated a week after the finding of plague rats in them and four of the 31 persons were inoculated—one two days after and three a week after plague rats were found in their houses. We may consider that all these persons were susceptible to plague infection but not a single one developed the disease.

## APPENDIX.

### ABSTRACT OF PLAGUE CASES IN DHAND.

NOTE:—A guinea-pig experiment consisted in allowing one or two guinea-pigs to run free for a night in the house in which a plague case occurred. The guinea-pigs were removed in the morning and the fleas caught on them were, in some instances, transferred to another guinea-pig in the laboratory. By a positive experiment is meant the death of either or both of these guinea-pigs from plague; by a negative experiment—that both remained healthy.



*Case A.*

Ruldoo, male, aet. 40, Hindu, water-carrier. Lives in house No. 352, Dhand; has not left the village for about 10 days. Attacked on 6th February—fever and left axillary bubo. Convalescent on 12th February:—bubo subsided without suppuration; material taken from the bubo on 9th February; films and cultures positive.

*Rats*—Live plague rats from adjoining house on 30th January.

Guinea-pig experiment—negative.

Connected previous case—nil.

*Case B.*

Mehr Singh, male, aet. 16, Hindu, dyer. Residence—No. 349, Dhand. Has not left the village for some months, except to go to Kasel occasionally. Attack—7th February—right axillary bubo and fever. Convalescent on 14th February; bubo subsided without suppuration. Material taken from the bubo on 9th February; films and cultures positive.

*Rats*—Live plague rats from adjoining houses, 347 and 350, on 27th January and 2nd February, and a dead plague rat from the latter house on 9th February. Four mummified rats from the back rooms of patient's house.

Guinea-pig experiment—positive.

Connected cases—nil.

*Case C.*

Khemi, female, aet. 26, Jat Sikh. Residence—340, Dhand. Arrived from another non-infected village 10 days previous to attack. Attack—12 February—left femoral bubo and fever. Convalescent on 23rd February; bubo subsided without suppuration.

*Rats*—A live plague rat from next house but one on 30th January. Patient was in the habit of going to this house.

Guinea-pig experiment—negative.

Connected cases—*vide* Cases E and L.

*Case D.*

Rhulla, male, aet. 30, Mahomedan, bhishti, employed as village watchman. Residence—498, Dhand. Attack—13th February—left femoral bubo and fever (104° F.). Died on 18th February. Material taken from the bubo on 17th February. Films and cultures positive.

*Rats*—A chronic plague rat from house No. 492 on 14th February; and a dead plague rat at No. 350 on 9th February, in which house patient assisted in the search for rats on that date.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case E.*

Tejo, female, aet. 9, Jat Sikh. Residence—upper storey of No. 338, Dhand. Has not left the village for two months. Attack on 13th February—right cervical bubo and fever (103·6°). Convalescent on 23rd February; bubo subsided without suppuration.

*Rats*—Nearest plague rat from house No. 342 on 30th January.

Guinea-pig experiment—negative.

Connected cases—probably visited Case C on 13/2/06, the day of her (E's) attack.

## Case F.

Santi, female, aet. 7, Jat Sikh. Residence—upper storey of No. 181, Dhand. Attack—16th February—double cervical buboes and fever ( $105\cdot2^{\circ}$  F.). Convalescent on 27th February. Bubo opened on 2nd March—pus sterile.

*Rats*—None in the vicinity till some weeks after the attack.

Guinea-pig experiment—negative.

Connected cases—nil.

## Case G.

Hazara, male, aet. 8, Jat Sikh. Has not left the village. Residence—No. 378, Dhand. Attack—21st February—no bubo; fever  $104^{\circ}$  F.; died 22nd February.

*Rats*—A dead plague rat found in lane not far from this house on 9th February.

Guinea-pig experiment—positive.

Connected cases—H, I and J.

## Case H.

Rami, female, aet. 70, Jat Sikh. Grandmother of Case G. Residence—No. 378, Dhand. Attack—24th February, fever  $105\cdot2^{\circ}$  F., stated she had no bubo, but did not allow examination of inguinal regions. Died on 27th February at midnight.

*Rats*—*vide* Case G.

Guinea-pig experiment—positive.

Connected cases—G, I and J.

## Case I.

Mangi, male, aet. 6, Jat Sikh. Residence—No. 378, Dhand, brother of Case G. Attack—24th February, fever  $104\cdot6^{\circ}$  F.; developed a right inguinal bubo on 26th February. Died 27th February.

*Rats*—  
Guinea-pig experiment } *vide* Case G.

Connected cases—H, G and J.

## Case J.

Nandi, female, aet. 70, Jat Sikh. Residence—No. 340, Dhand. Lives with Case C, who is her daughter-in-law. Frequently went to house No. 378 during the illness of Cases G, H and I, Case H being her sister, and attended the funeral party of the latter. Attack—night of 27th February, fever  $103^{\circ}$ ; states she had no bubo, but did not allow examination—Fever continued and remained high and patient became delirious on 9th March and died on 12th March.

*Rats*—  
Guinea-pig experiment } *vide* Case G.

Connected cases—G, H, and I. *Vide supra*.

## Case K.

Taboo, male, aet. 10, Jat Sikh. Residence—upper storey of No. 335, Dhand. House adjoins No. 378, where Cases G, H, and I occurred. Attack—5th March, fever  $103^{\circ}$  F., and right inguinal bubo; convalescent on 13th March. Bubo opened on 15th March; culture from pus gave growth of *Staphylococcus*, no *B. pestis*.

*Rats*—Nearest plague-infected rat in lane near house No. 377 on 9th February, but house No. 378 proved by guinea-pig experiment to be infective on 24th February.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case L.*

Waryam Singh, male, aet. 60, Jat Sikh. Residence—159, Dhand; has not left Dhand for some weeks, except to visit Mianpur village on 21st March. Attack—22nd March, fever; left inguinal bubo; vomiting and thickness of speech. Died on 23rd March at midnight.

*Rats*—A dead plague rat from neighbouring house, No. 60, on 18th March.

Guinea-pig experiment—negative.

Connected cases—Case L took medicine to Case C on a few occasions during the latter's illness.

*Case M.*

Imambibi, female, aet. 20, Mahomedan Teli. This woman came to Dhand from Bachiwind, a non-infected village in the Ajuala Tahsil on the 22nd March. After arrival in Dhand she lived with her brother in house No. 401 which had been closed prior to her arrival. Attack—26th March, left femoral bubo and fever. Died on 31st March.

*Rats*—A dead plague-infected rat was found in the room adjoining living room on 29th March.

Guinea-pig experiment—positive.

Connected cases—nil.

*Case N.*

Umri, female, aet. 40, Mahomedan Teli. Residence—39, Dhand. Attacked on 28th March, died 29th March. This case was not seen till after death, when no bubo was found on examination. She is said to have had fever and delirium with blood-tinged expectoration.

*Rats*—None in vicinity.

Guinea-pig experiment—positive.

Connected cases—nil.

*Case O.*

Tabo, female, aet. 10, Jat Sikh. Residence—603, Dhand (in new village). This girl had returned from a visit to her uncle in Chhina village in the Ajuala Tahsil about the 16th March. There is no evidence that Chhina was infected. She frequently went to house 429, Dhand where she took sewing lessons and is said to have swept out that house on 27th March. Attack—29th March, fever and left femoral bubo; convalescent on 7th April; bubo subsided without suppuration.

*Rats*—None at or near her residence prior to attack, but plague rats at houses Nos. 583 and 618 in the vicinity on 1st April and 6th April.

Guinea-pig experiment—none at residence; negative in house No. 429.

Connected cases—nil.

*Case P.*

Isar Singh, male, aet. 45, Jat Sikh. Residence—456, Dhand. Came to this house about 15 days before attack from a non-infected part of the village. Attack—31st March; fever on 1st April; a painful swelling developed below outer half of right clavicle, which later became hard with oedema of overlying skin. Died on 4th April. Material taken from the swelling on 3rd April—smear indefinite; culture positive.

*Rats*—A dead plague rat in lane adjoining residence on 13th March.

Connected cases—nil.

*Case Q<sup>1</sup>.*

Rukki, female, aet. 25, Chuhra (sweeper). Residence—225, Dhand. Attack—4th April; fever and right femoral bubo; marked slurring of speech; convalescent on 13th April; bubo subsided without suppuration.

*Rats*—None in house or vicinity.

Guinea-pig experiment—*vide* Case T.

Connected cases—Case T.

*Case R.*

Abdul Rahman, male, aet. 9, Mahomedan Kamboh. Brought to house No. 85, Dhand on the morning of 6th April suffering from plague. Attacked during night of 5th April in Chabal village—right femoral bubo and fever; convalescent on 13th April; bubo subsided without suppuration.

*Rats*—History of dead rats at residence in Chabal; none in or near residence at Dhand.

Connected cases—Case W.

*Case S.*

Dhunda, male, aet. 5, Mahomedan Teli. Residence—3, Dhand. Patient is said to have gone to house No. 39 (*vide* under Case N) frequently prior to attack. Attack—7th April; fever and right inguinal bubo. Died on 13th April.

*Rats*—Live infected rats from houses 185 and 186 prior to attack.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case T.*

Santi, female, aet. 12, Chuhra. Residence—225, Dhand with Case Q. Attack—fever on 7th and left axillary bubo on 8th April. Died on 11th April.

*Rats*—None in house or vicinity.

Guinea-pig experiment—positive on 10/4/06.

Connected cases—Q attacked in same house on 4th April.

*Case U.*

Asso, female, aet. 16, Hindu, water-carrier. Residence—622, Dhand. Attack on 10th April, fever 103·8° F. and left axillary bubo. Convalescent on 13th April; bubo subsided without suppuration.

*Rats*—Dead plague rats from house No. 618, in adjoining courtyard, on 6th April.

Guinea-pig experiment—negative.

*Case V.*

Bhagat Singh, male, aet. 20, Hindu, water-carrier, employed in a native regiment at Bannu. Came to his home on leave on 15th March. Residence—622, Dhand, with his sister Asso (Case U). Attack—10th April; fever and left femoral bubo the next morning. Unconscious on 11th and 12th. Died on the evening of 12th April.

*Rats*—*vide* Case U.

Guinea-pig experiment—*vide* Case U.

Connected case—Case U.

<sup>1</sup> Marked R in Map I, in northernmost part of village.

*Case W.*

Abdul Gani, male, aet. 6, Mahomedan Kamboh. Came from Chabal on 6th April to stay in house No. 85, Dhand, where his brother, Case R, had been brought on the 5th April suffering from plague. Attack—fever on 12th April; temperature 102° F. on 13th and 14th, with intermission on the 15th and 16th when patient appeared well. On the 17th he developed femoral buboes on both sides and a swelling behind the angle of right jaw and slight fever (99° F. to 100° F.). Convalescent on 21st—all the buboes subsided without suppuration.

*Rats*—*vide* Case R.

Guinea-pig experiment—*vide* Case R.

Connected case—Case R. *Vide supra.*

*Case X.*

Gogi, male, aet. 20, Mahomedan, water-carrier. Residence—587, Dhand. Attack—fever on 14th April; on 17th temperature 102·2° F. with a small left inguinal bubo. Convalescent on 21st April; bubo subsided without suppuration.

*Rats*—Plague rats from houses Nos. 583 and 618 in vicinity on 1st April and 6th April.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case Y.*

Jugo, female, aet. 30, Hindu, water-carrier. Residence—589, Dhand. Patient went to see Cases U and V in house No. 622 on 11th April and attended the latter's funeral party on 13th April. Attack—right axillary bubo and fever on 14th April—convalescent on 17th; bubo subsided without suppuration.

*Rats*—Plague rats. *Vide* under Case X.

Guinea-pig experiment—negative.

Connected cases—U and V. *Vide supra.*

*Case Z.*

Parsino, female, aet. 12, Jat Sikh. Residence—122, Dhand. This girl left Dhand on or about the 11th April to stay with a relative in Modhe, a village in the Tarn Taran Tahsil. Attack—about 16th April in Modhe, with fever. Patient was brought back by her father to Dhand on the 20th and died on the 21st April. The case was not seen till after death and examination for buboes was not permitted, but relatives said that patient had a small right femoral bubo when brought back to Dhand. No plague was reported from Modhe till 30th April when some cases occurred at a distance from the quarter where patient stayed.

*Rats*—No history of dead rats in or near the house in Modhe where patient stayed. Infected rats from house No. 137, Dhand, which immediately adjoins No. 122, on 31st March and 3rd April.

Connected cases—nil.



*Plague in Dhand**Case AA.*

Bibi, female, aet. 20, Mahomedan Kamboh. Residence—583, Dhand. Attack—19th April, right femoral bubo and fever. Died on 22nd April.

*Rats*—Live plague rat from residence on 1st April and a dead plague rat from adjoining house on 6th April.

Guinea-pig experiment—positive.

Connected cases—nil.

*Case BB.*

Luchmi, female, aet. 25, Hindu, water-carrier. Residence—588, Dhand. Attack—20th April, fever followed by left femoral bubo. Died on 26th April.

*Rats*—*vide* Case AA.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case CC.*

Bhega, female, aet. 30, Mahomedan Teli. Residence—No. 2, Dhand. Attack—21st April; fever, no bubo found, but complete examination not allowed. Blood-tinged expectoration on 24th. Patient died on 25th April.

*Rats*—None in house or vicinity.

Guinea-pig experiment—(on 25th April) negative.

Connected cases—nil.

*Case DD.*

Jawali, female, aet. 7, Chuhra. Residence—220, Dhand. Attack—fever on 23rd April, temperature on 24th 102·6° F.; got double inguinal buboes on 28th and a right axillary and double submaxillary buboes on 29th. Fever continued and patient died on 13th May.

*Rats*—None in house or vicinity.

Guinea-pig experiment—positive.

Connected cases—nil.

*Case EE.*

Jhando, female, aet. 21, Mahomedan, bhishti. Residence—386, Dhand. Attack—1st May; fever 102·2° F.; died 4th May. Examination for buboes not permitted and relatives said there were none present.

*Rats*—Plague rats from house adjoining and lane adjoining on 21st April.

Guinea-pig experiment—negative.

Connected cases—nil.

*Case FF.*

Sultano, female, aet. 22, Mahomedan, bhishti. Residence—387, Dhand. Attack—2nd May; fever and delirium. No examination for buboes permitted. Died 4th May.

*Rats*—*vide* under Case EE.

Guinea-pig experiment—negative.

Connected cases—nil.

IV. OBSERVATIONS WHICH HAVE SPECIAL REFERENCE  
TO THE VILLAGE OF KASEL.

- I. Introduction.
  - (1) Situation, etc.
  - (2) Census results.
- II. Previous epidemics of plague.
- III. The plague epidemic of 1906.
  - (1) Period before the epidemic.
  - (2) Period during the epidemic.
    - (a) Severity and duration of epidemic.
    - (b) Clinical features of the cases.
      1. Type of the cases.
      2. Situation of the bubo.
      3. Mode of onset.
      4. Sex incidence.
      5. Age incidence.
      6. Caste incidence.
      7. Case mortality.
    - (c) Distribution of the cases amongst the houses.
    - (d) Contact with previous cases.
- IV. The epizootic.
  - (1) Period before the epizootic.
  - (2) Period during the epizootic.
  - (3) Period after the epizootic.
  - (4) Origin of the epizootic.
- V. Relation between the epizootic and the epidemic.
  - (1) Relation in time.
  - (2) Relation in place.
  - (3) Interval between the finding of dead plague rats in houses and the occurrence of the first plague case in them.
  - (4) Finding of plague-infected rats in houses not always followed by plague cases.
  - (5) The influence of evacuation of houses, in which dead plague rats were found, on the incidence of plague on the occupants.

I. INTRODUCTION.

1. *Situation.*

Kasel is situated about three-quarters of a mile south-west of Dhand and hence is at a distance of about nine miles from Amritsar city. It has communication by cart roads with Amritsar, Tarn Taran, Ghandwind, Dhand and several other villages. Unlike Dhand, part of the area on which Kasel is situated is raised considerably above the surrounding country. This is due to the present village having been built on the

ruins of previous villages. The highest point corresponds to houses 235, 236 near the middle of the village. From this point the ground slopes down in all directions. The peripheral parts of the village are built on level ground. The area of Kasel is roughly 40 acres.

## 2. *Census results.*

The population, according to our census, was 3938, and the density of population roughly 100 per acre.

The number of occupied houses is 806. 320 of these contain a single room, 276 two rooms, and 210 more than two rooms. 77 of the houses are two-storied, the upper storey usually consisting of a single room. Of the occupied houses only 25 are brick and mortar structures, the remainder being mud houses of the type already described.

The inhabitants are of the same castes as those of Dhand and follow similar occupations. Adopting the same classification as was done in the latter village, we find that the population is made up of:

Sikhs and Hindus	.	.	.	.	1419
Mahomedans	.	.	.	.	2063
Chuhra	.	.	.	.	456

It will be noticed that the Sikhs and Hindus bear a smaller proportion to the total population than in Dhand.

## II. PREVIOUS EPIDEMICS OF PLAGUE.

Plague first appeared in Kasel in October 1902, the first case recorded being a sweeper, who apparently contracted the disease in another village and died in Kasel on 13th October 1902. The epidemic which followed lasted till the second week of January 1903. There were 487 attacks with 225 deaths.

With the exception of a few imported cases the village remained free from plague until the last week in December 1903, when the disease again appeared as an epidemic. This second epidemic lasted till the end of April 1904. The number of attacks was 386 and of deaths 273. After another plague-free interval of nine months, the disease reappeared early in February 1905. The epidemic of 1905 lasted till the end of April and consisted of 342 attacks with 210 deaths. The last recorded death from plague in 1905 occurred on the 28th of April. We could not determine the source of origin of the epidemics of 1904 and 1905.

In Kasel, as in Dhand, all the evidence went to show that during the three epidemics dead rats were found in connection with plague cases.

### III. THE PLAGUE EPIDEMIC OF 1906.

#### 1. *Period before the epidemic.*

The last death from plague in 1905 occurred on the 28th April. From the end of November, when the Commission commenced its observations in the village, till the 12th March, no case of plague came to light. On the latter date a child of the Chuhra caste, who had left Kasel about twelve days previously, was brought back to the village suffering from plague and died on the 14th March (*vide* Case 1, Appendix). No further case occurred till the 5th April.

#### 2. *Period of the epidemic.*

##### (a) *Severity and duration of epidemic.*

The epidemic of 1906 consisted of 75 cases. The date of attack of the first case was the 5th April and of the last case the 6th July. A list of the cases and a short description of each will be found in the Appendix.

##### (b) *Clinical features of the cases.*

1. *Type of the cases.* Of 71 cases, in which a complete examination was made, 68 were bubonic, the other three having no buboes. One of the three latter cases (No. 11) had primary plague pneumonia. Among the 68 bubonic cases were two with recent abrasions (Nos. 5 and 8), and two with carbuncles (Nos. 33 and 65), in the drainage area of the affected glands. One bubonic case (No. 13) developed a secondary plague pneumonia. Four cases, viz. Nos. 6, 31, 53 and 67, were not completely examined, so that the presence of a bubo could not be excluded. Of these, No. 6 was not seen till just before death, but from the history it is probable that it was a case of plague pneumonia: case No. 31 had a phlyctenule on the right ankle and case 67 had cough and rusty sputum in which abundant *B. pestis* were present.

2. *Situation of the bubo.* The situation of the bubo in the 68 bubonic cases examined was as follows.

Femoral . . . . .	52
Axillary . . . . .	8
Cervical . . . . .	4
Femoral and Cervical . . . . .	3
Axillary and Cervical . . . . .	1

3. *Mode of onset.* In 54 cases pain in the gland, with or without swelling, was amongst the earliest symptoms. In seven cases the bubo developed on the day following the appearance of other symptoms; in four cases after two days, in two cases after three days and in one case after eight days.

4. *Sex incidence.* Table XXII shows the relative proportion of males and females attacked. It will be seen that the incidence on females was more than twice as high as that on males.

TABLE XXII.

*Showing the incidence of attacks on males and females in Kasel.*

Sex	Total population	Plague attacks	Incidence per 1000
Males	2129	25	12
Females	1809	50	28

TABLE XXIII.

*Showing the relative incidence of attacks on persons at different age periods in Kasel.*

Age period	Total population	Plague attacks	Incidence per 1000
0—5 years	836	5	6
6—10 „	477	14	29
11—20 „	860	14	16
21—40 „	1144	33	29
Over 41 „	621	9	14

5. *Age incidence.* Table XXIII shows the relative incidence on persons at different age periods. It will be seen that the very young were apparently less liable to attack than persons of other ages.

6. *Caste incidence.* The plague incidence on the three classes into which we have grouped the inhabitants is summarised in Table XXIV. This table shows that in Kasel the sweepers, who represent the lowest classes of the village community, suffered more than the Hindus or Mahomedans. In Dhand, it will be remembered, the incidence was greatest on the Hindus.

TABLE XXIV.

*Showing the plague incidence on different castes in Kasel.*

Caste	Total population	Plague attacks	Incidence per 1000
Sikhs and Hindus	1419	26	18
Mahomedans	2063	32	15
Chuhra (menials)	456	17	37



7. *Case mortality.* Out of 75 cases, 41 or almost 55 % ended fatally. The longest interval between attack and death was 10 days and the shortest one day. The average for 38 fatal cases in which the interval was accurately determined was a little over four days.

(c) *Distribution of cases among houses.*

The 75 cases inhabited 67 houses. One house furnished three cases, six houses furnished two cases each and 60 houses a single case each. All the cases were treated in their own homes during the whole period of their illness.

In the houses which furnished single cases the number of contacts was 273, of whom only 18 had been inoculated against plague. In other words out of 255 presumably susceptible persons who came into close contact with plague cases none were attacked.

(d) *Contact with previous cases.*

Out of 75 cases it was ascertained that 53 or 70 % did not, prior to attack, come into contact with other plague cases (*vide* Appendix, cases 2 to 4, 7, 10, 12 to 15, 17, 19, 20, 22 to 31, 34 to 37, 42 to 55, 58 to 61, 63, 65, 66, 68 and 70 to 75). Eleven cases (15 %) were found to have had contact with previous cases prior to becoming ill (Appendix, cases 9, 11, 16, 32, 33, 39, 41, 43, 57, 64 and 69). With regard to the remaining 11 cases (15 %) (Appendix, cases 1, 5, 6, 8, 18, 21, 38, 40, 56, 62 and 67), we could not determine whether or not such contact had taken place.

We may, however, conclude that the majority of cases cannot have received their infection from a previous case.

#### IV. THE EPIZOOTIC.

##### 1. *Period before the epizootic.*

(From 29th November, 1905, to 1st April, 1906.)

(Map 2.)

During this period 2510 *Mus rattus* were examined at the laboratory; 2460 of these had been trapped, and 50 were found dead. It is to be noted that trapping was suspended in Kasel from 1st January, 1906, to 20th February. None of the rats examined were found to be suffering from acute plague, but two live rats were found with chronic abscesses in the spleen from which *B. pestis* was recovered. The first of these chronic plague rats was trapped on 9th December and the last on 12th December (*vide* Map 2).

*2. Period of epizootic.*

(From 2nd April, 1906, to 17th July, 1906.)

(Maps 1 and 3—16. Table XXV.)

During this period 1254 rats were examined, of which number 911 were caught alive and 343 were found dead. Plague was identified in 27 live rats and in 226 dead rats. 89 of the dead rats were found in such an advanced stage of putridity that a diagnosis could not be made. Many of these were found in houses in association with proved plague rats and there can be little doubt that most of them died of plague.

The first acute plague rat was found in a dying condition in house No. 122 on the 2nd April (*vide* Map No. 1, Serial No. 1 and Table No. 24). The occupants informed us that about five days previously they had noticed a bad smell in the house which they attributed to a dead rat. The next two plague rats were found dead in house No. 114 which is in the vicinity of No. 122 (*vide* Map No. 1, Serial No. 2 and Table No. 24). These first three plague rats exhibited, on post-mortem examination, buboes and other lesions typical of acute plague and, microscopically, abundant *B. pestis* were present in the buboes and heart-blood.

The further progress of the epizootic can best be followed by referring to Map 1 and Table XXV and the series of weekly Maps Nos. 3—16.

The latter series shows well the gradual extension of the epizootic outwards from the original focus of infection, in the same rather irregular manner as was noted in the case of Dhand. It will be noticed, however, that this extension was not associated with the simultaneous disappearance of the epizootic from the more central parts. On the contrary infected rats continued to be found in the vicinity of the original focus of infection till the sixth week of the epizootic period, by which time the epizootic had spread over a large part of the village.

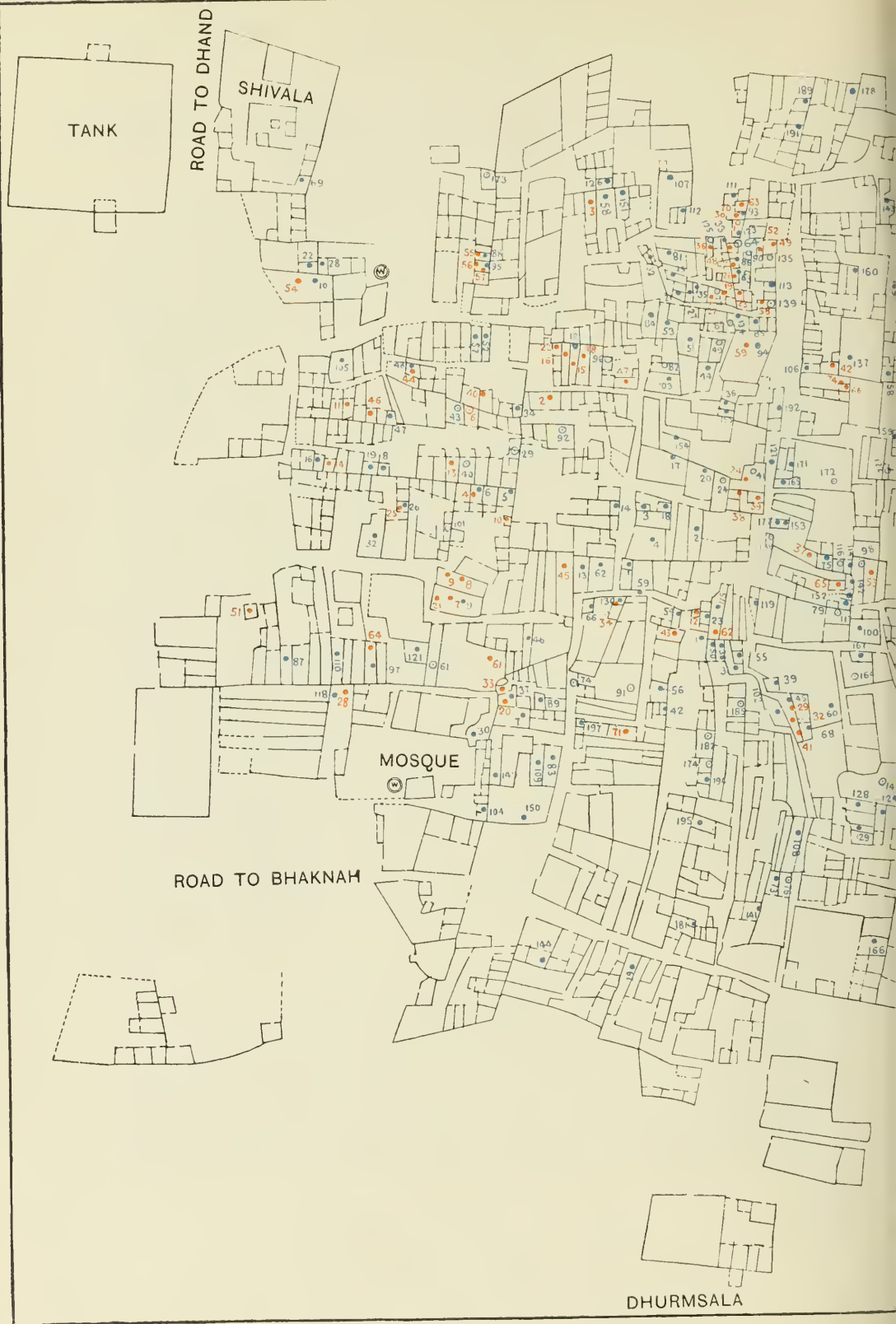
*3. Period after the epizootic.*

(18th July—5th December, 1906.)

The last acute plague rat was found on the 17th July, 1906. From the 18th July to the conclusion of the observations in Kasel on the 5th December, 1906, 1468 rats were examined, of which 1449 were trapped alive and 19 were found dead. While no rat with acute plague was found during this period seven rats suffering from chronic plague

MAP 1

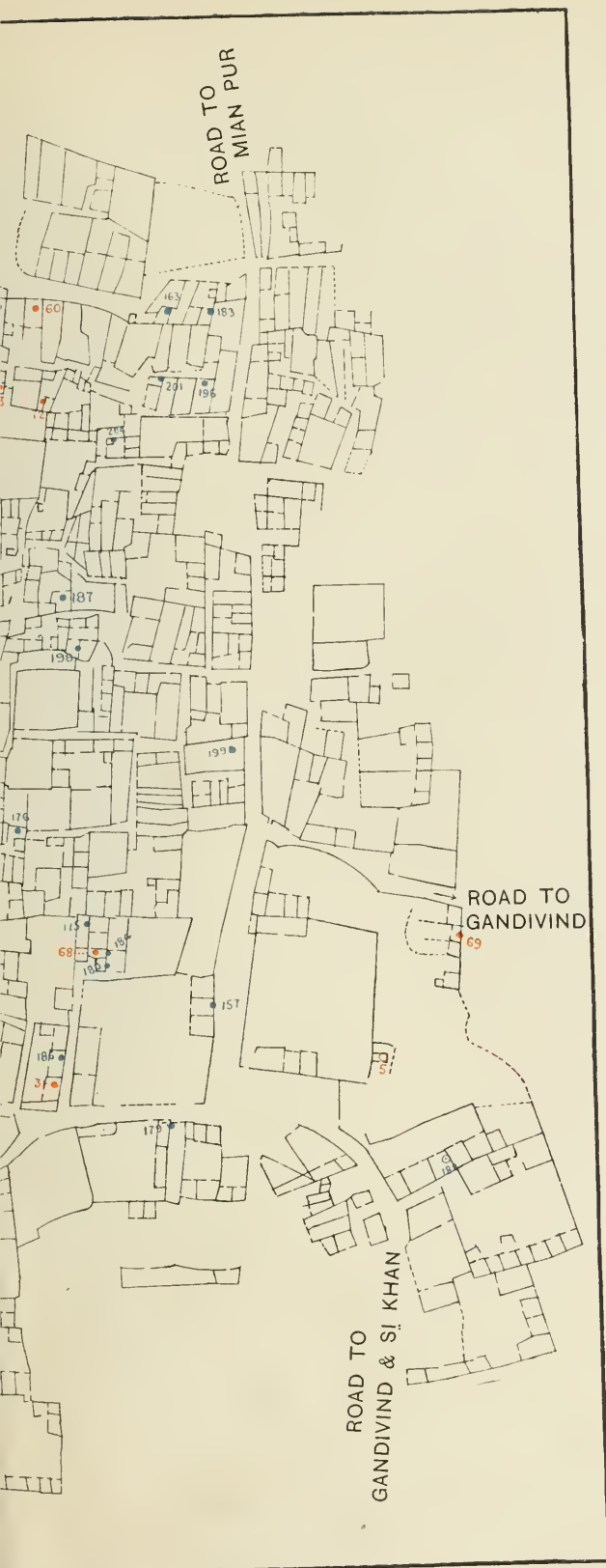
KASEL VILLAGE



## KASEL VILLAGE

100 feet

- Human plague case (serial number)
- Plague infected rat or rats (serial number)
- Putrid or mummified rat or rats



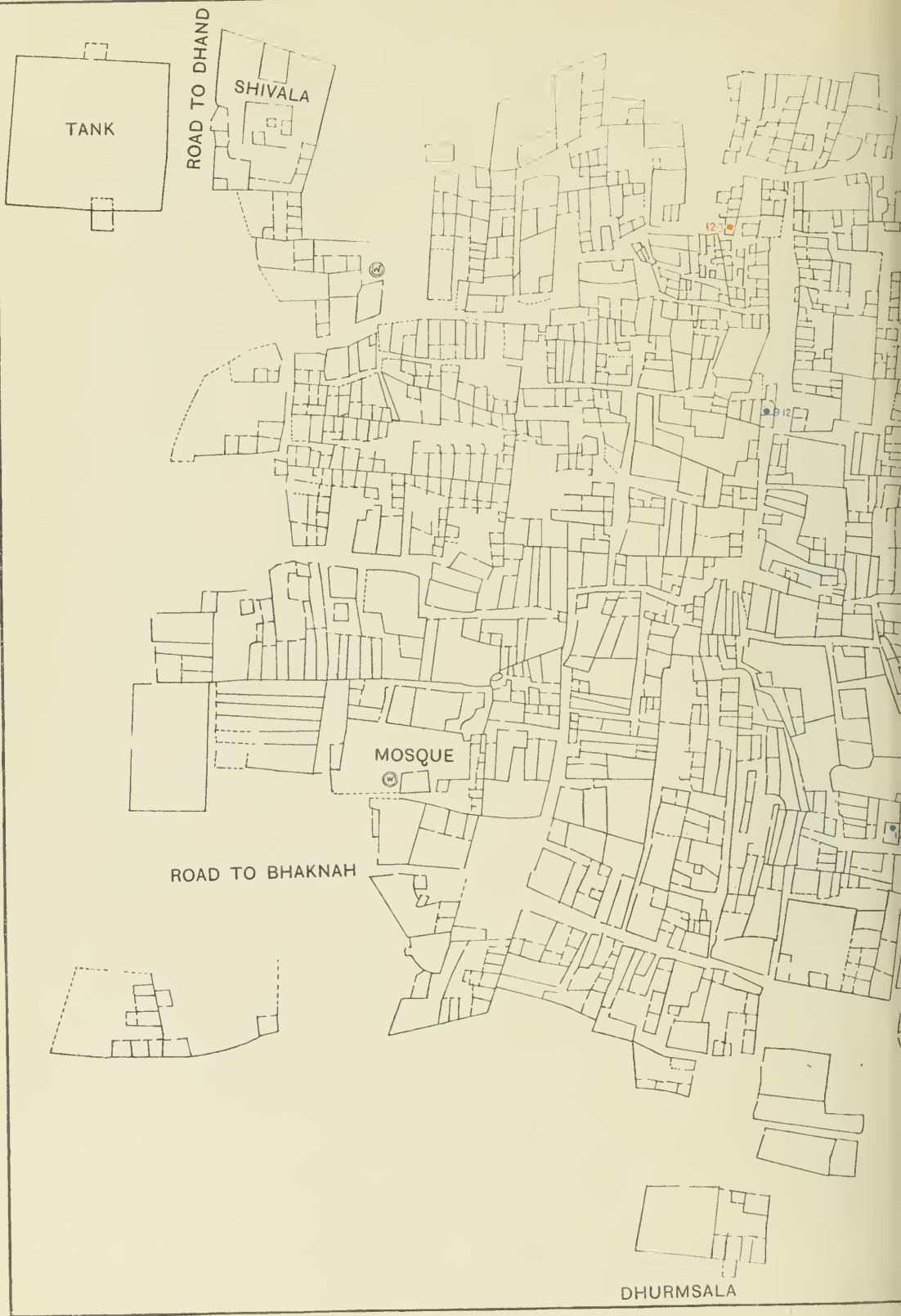




## MAP 2

### KASEL VILLAGE

Period before the epizootic,  
29 Nov. 1905 to 1 April, 1906

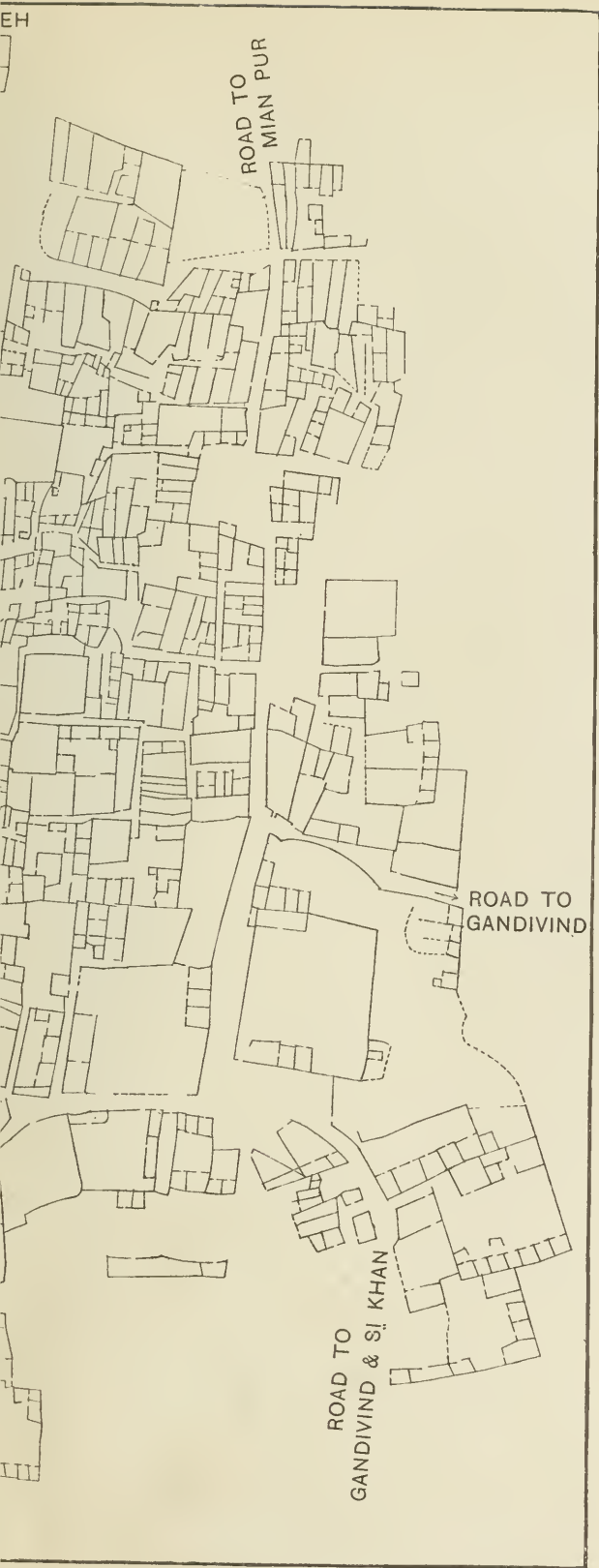


# KASEL VILLAGE

Period before the epizootic,  
29 Nov. 1905 to 1 April, 1906

100 feet

- Human plague case (imported)
- Chronic plague rats (date)



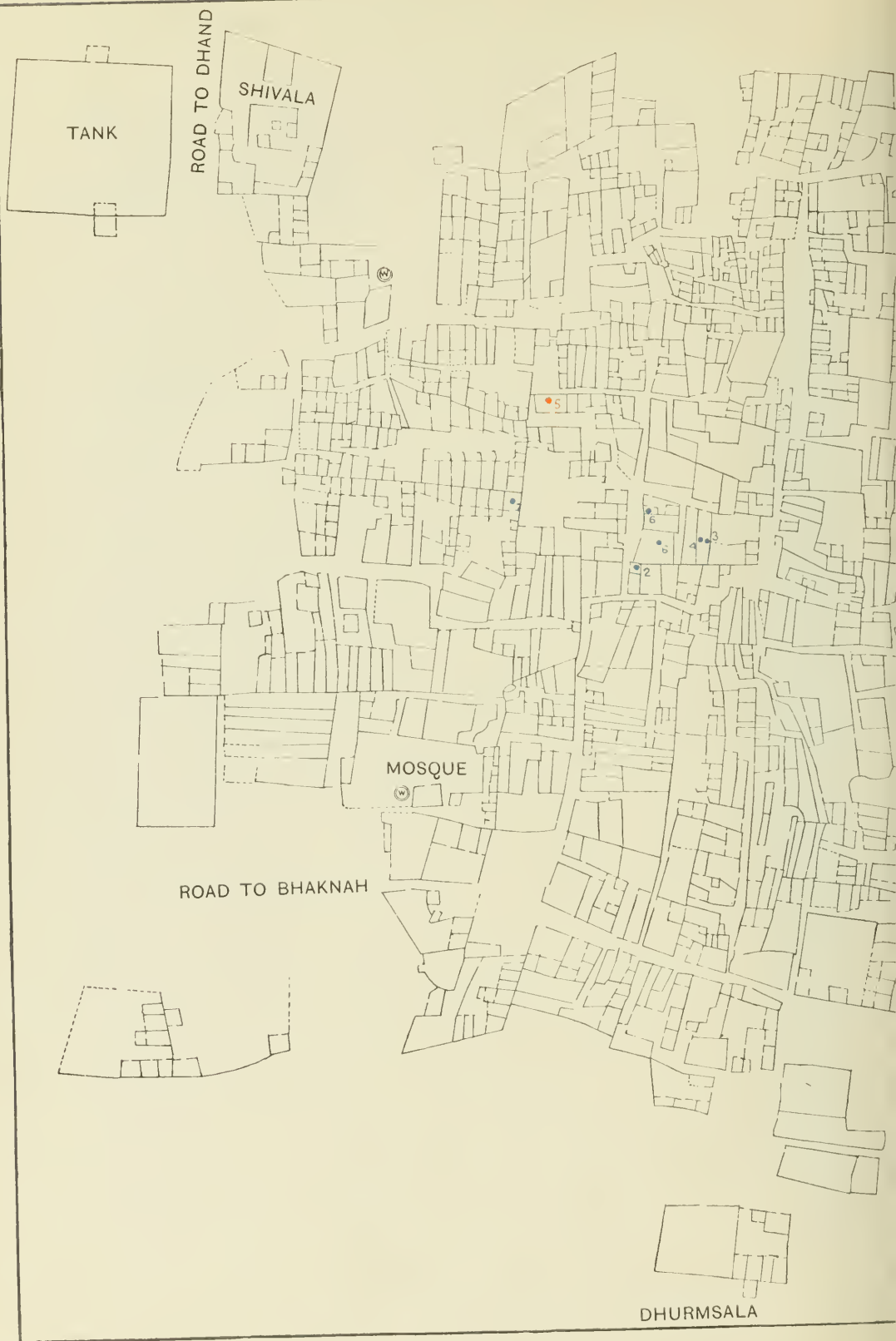




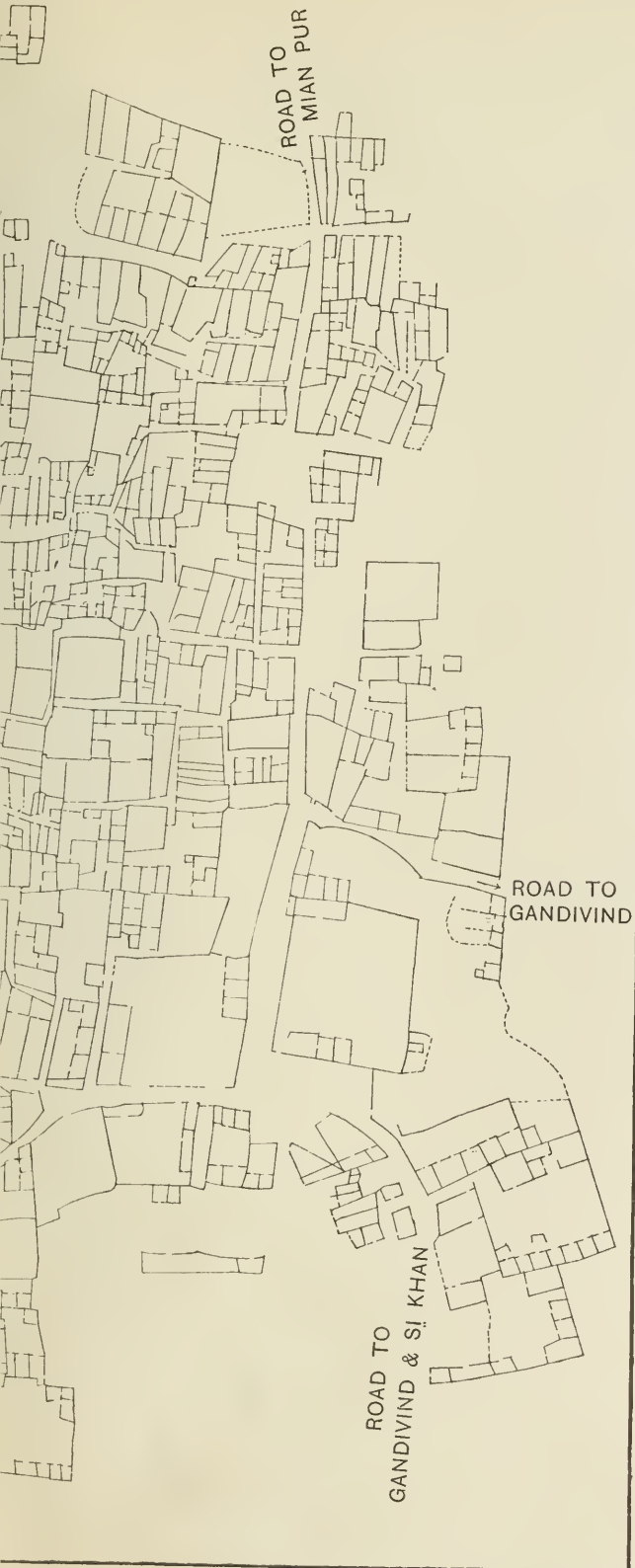
## MAP 3

### KASEL VILLAGE

First week of epizootic,  
2 April, 1906 to 8 April, 1906



TAKEH



MAP

# KASEL VILLAGE

First week of epizootic,  
2 April, 1906 to 8 April, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)

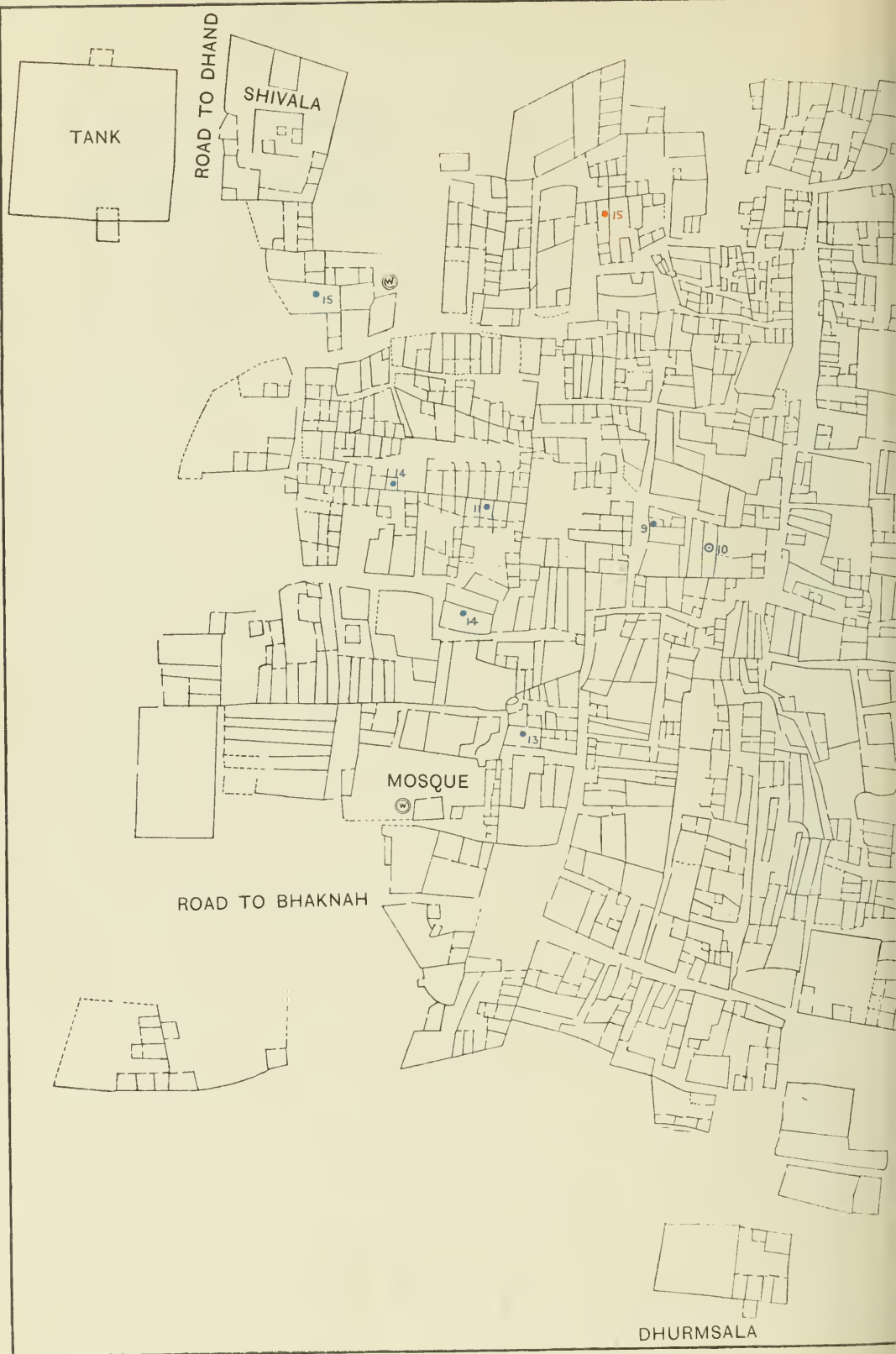


## MAP 4

### KASEL VILLAGE

Second week of epizootic,  
9 April, 1906 to 15 April, 1906





## KASEL VILLAGE

Second week of epizootic,  
9 April, 1906 to 15 April, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat

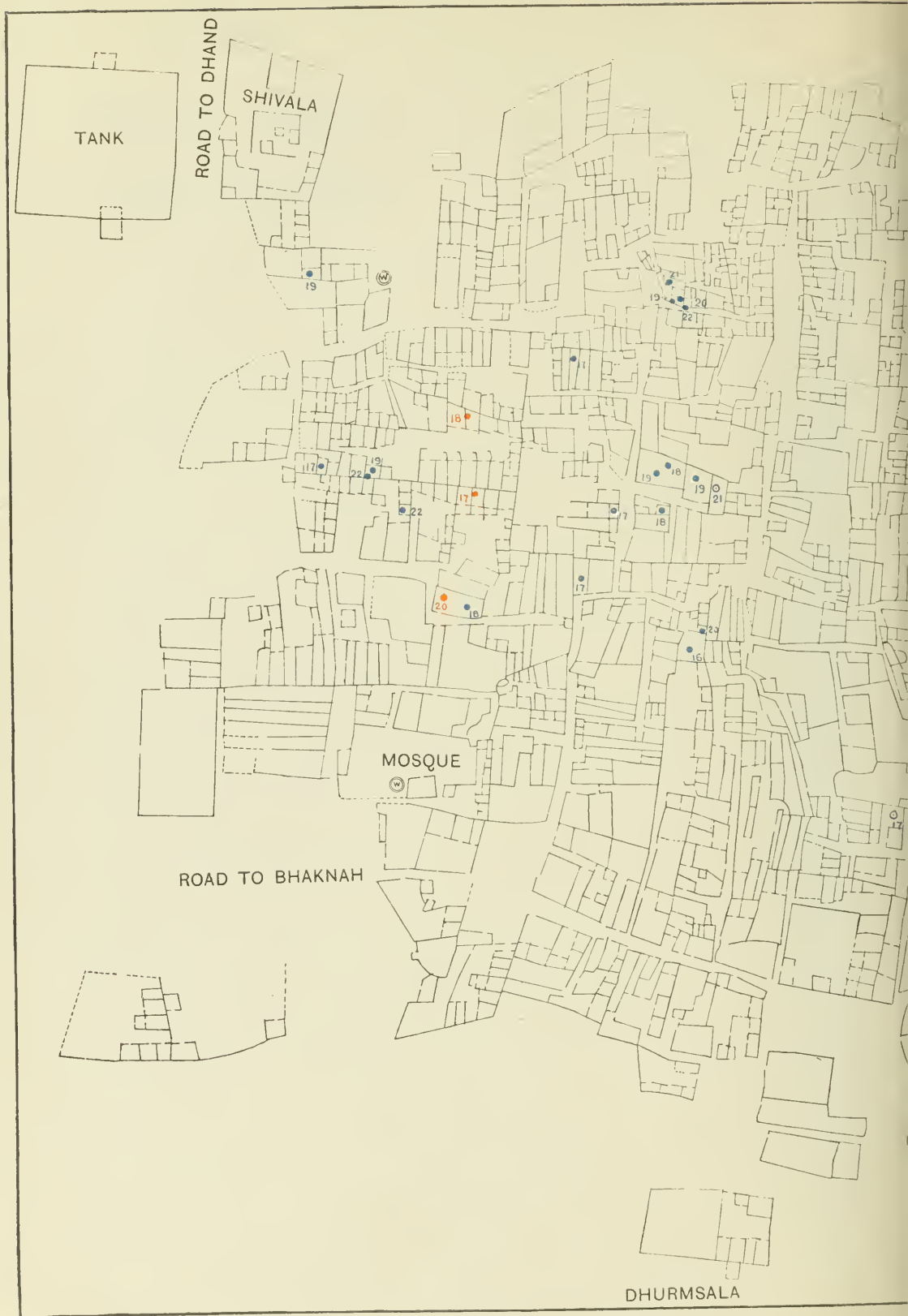




## MAP 5

### KASEL VILLAGE

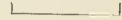
Third week of epizootic,  
16 April, 1906 to 22 April, 1906





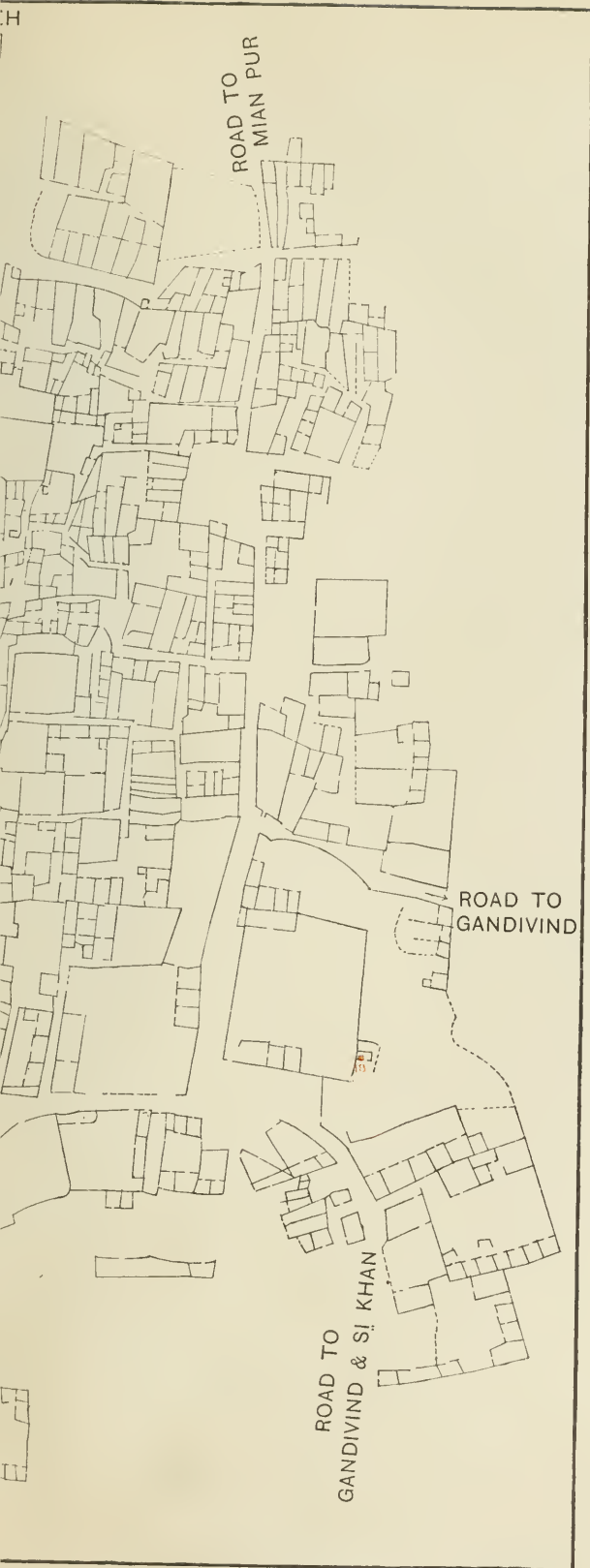
# KASEL VILLAGE

Third week of epizootic,  
16 April, 1906 to 22 April, 1906



100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat

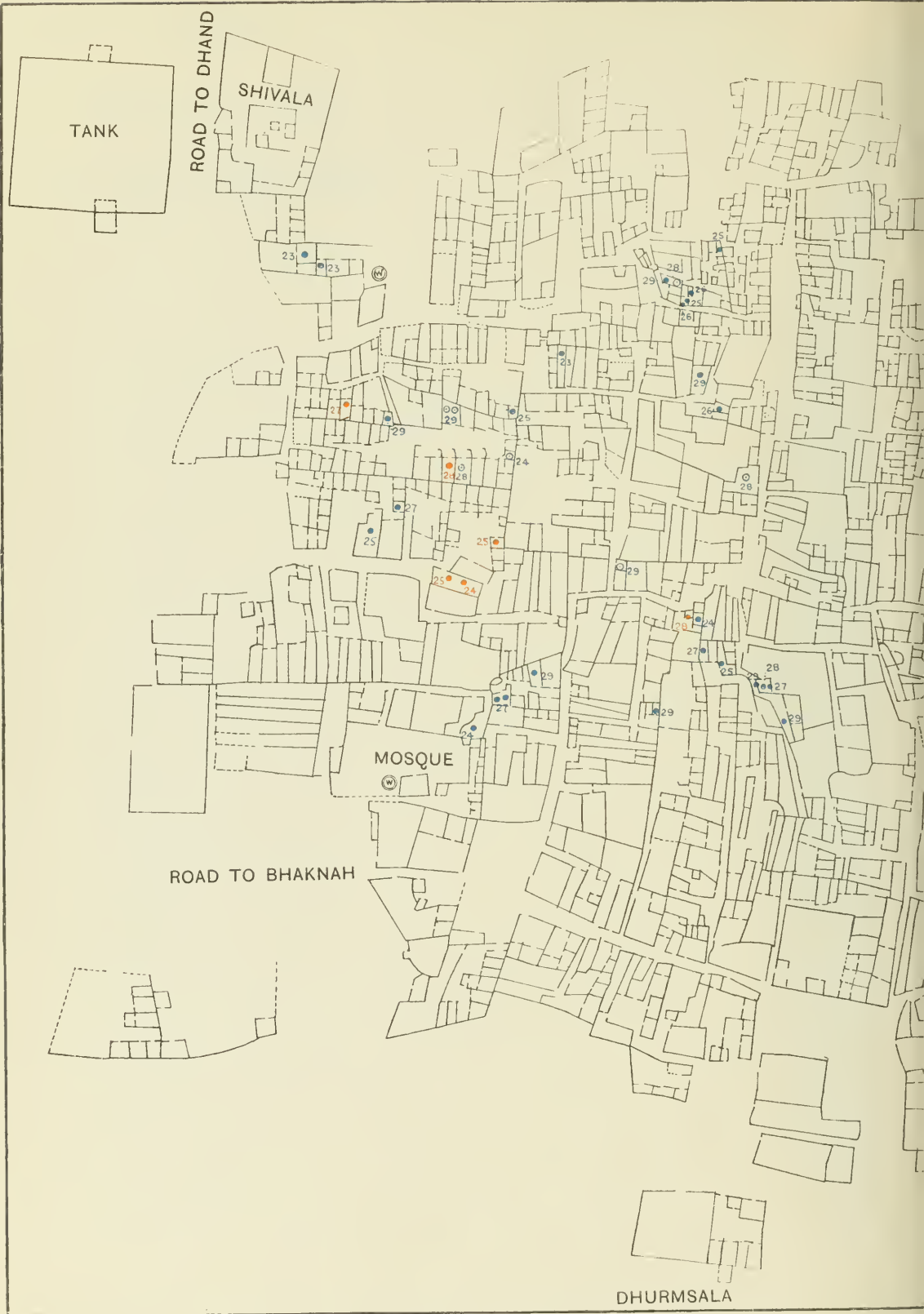




## MAP 6

### KASEL VILLAGE

Fourth week of epizootic,  
23 April, 1906 to 29 April, 1906

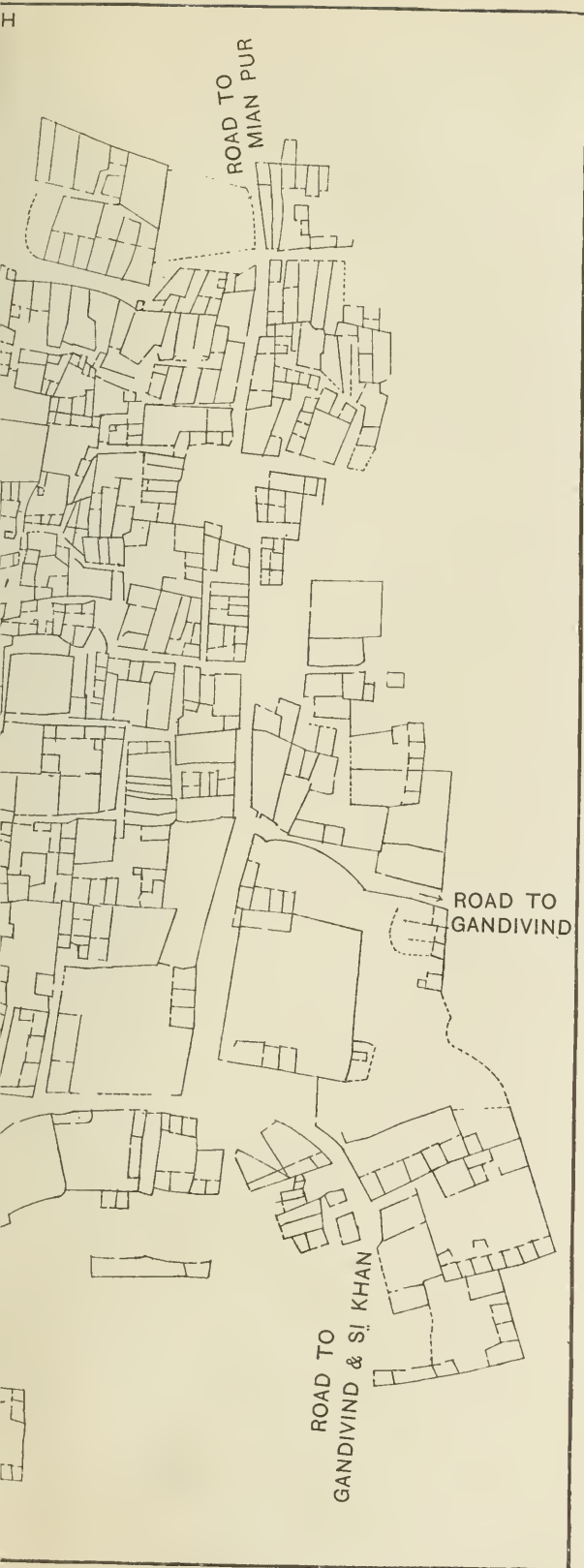


# KASEL VILLAGE

Fourth week of epizootic,  
23 April, 1906 to 29 April, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



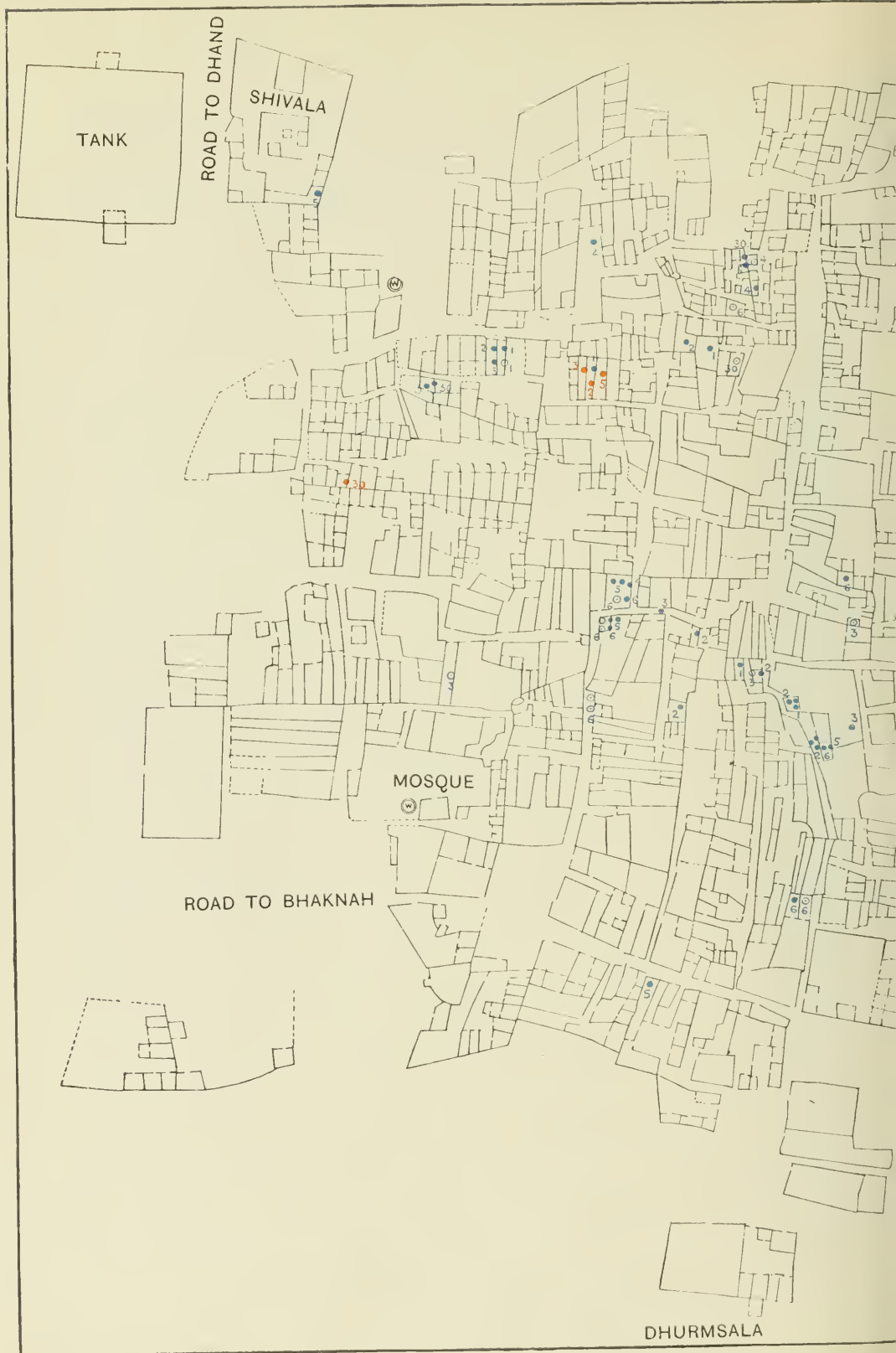




MAP 7

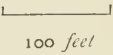
KASEL VILLAGE

Fifth week of epizootic,  
30 April, 1906 to 6 May, 1906

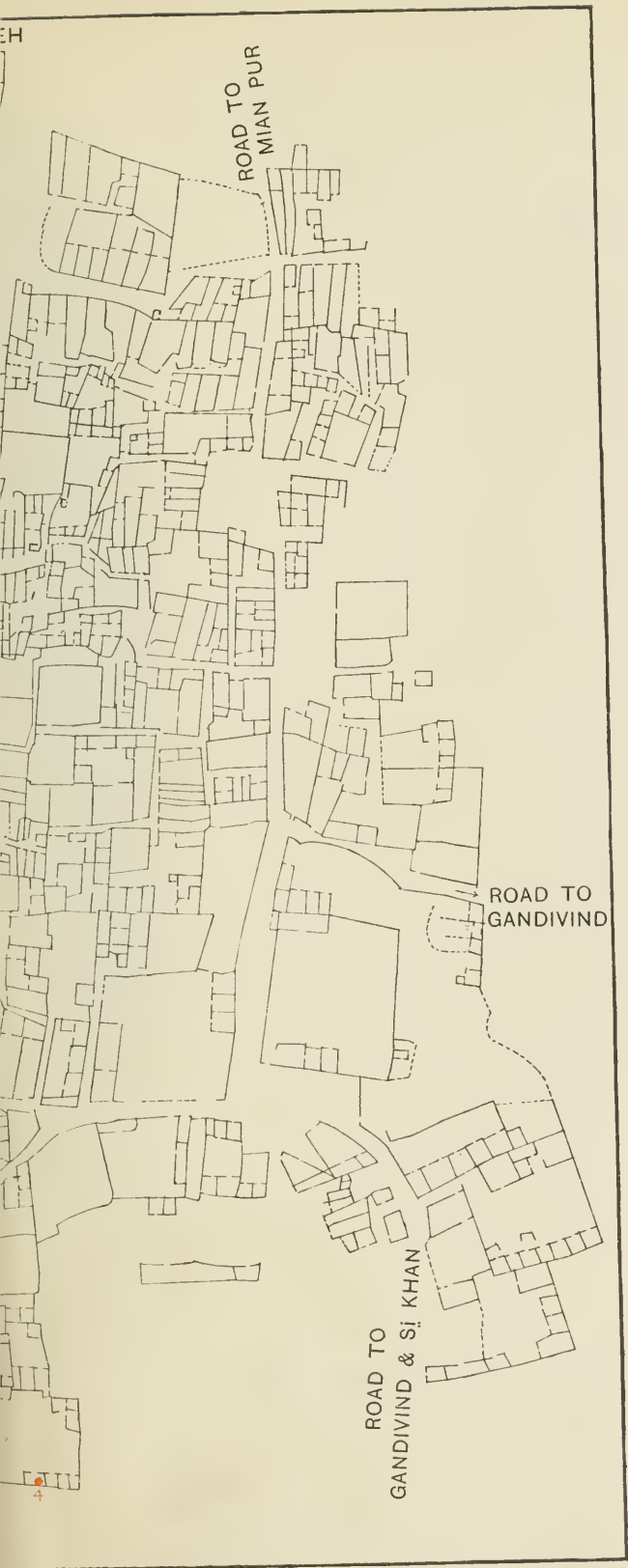


# KASEL VILLAGE

Fifth week of epizootic,  
30 April, 1906 to 6 May, 1906



- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



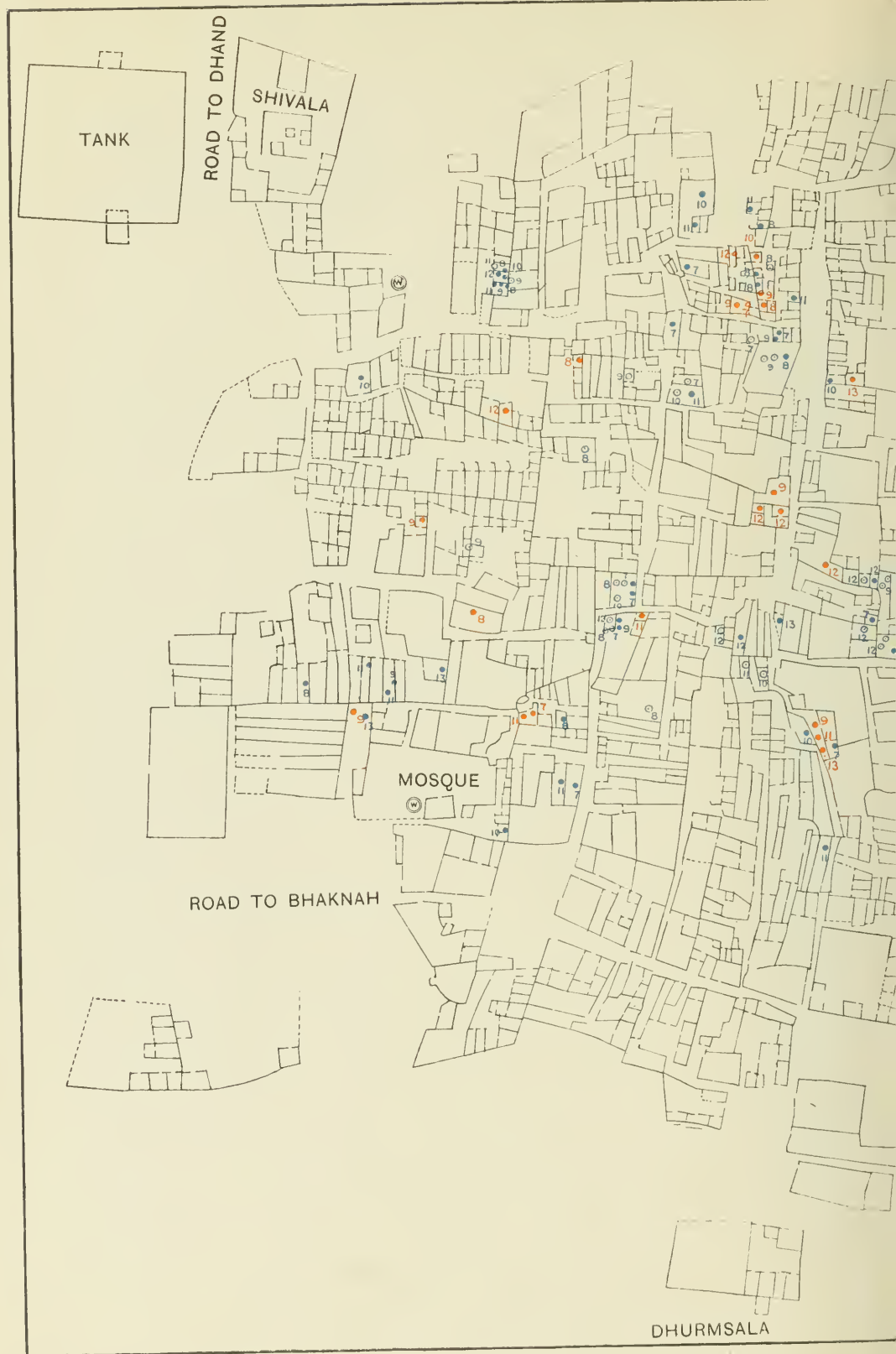




## MAP 8

### KASEL VILLAGE

Sixth week of epizootic,  
7 May, 1906 to 13 May, 1906



# KASEL VILLAGE

Sixth week of epizootic,  
7 May, 1906 to 13 May, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



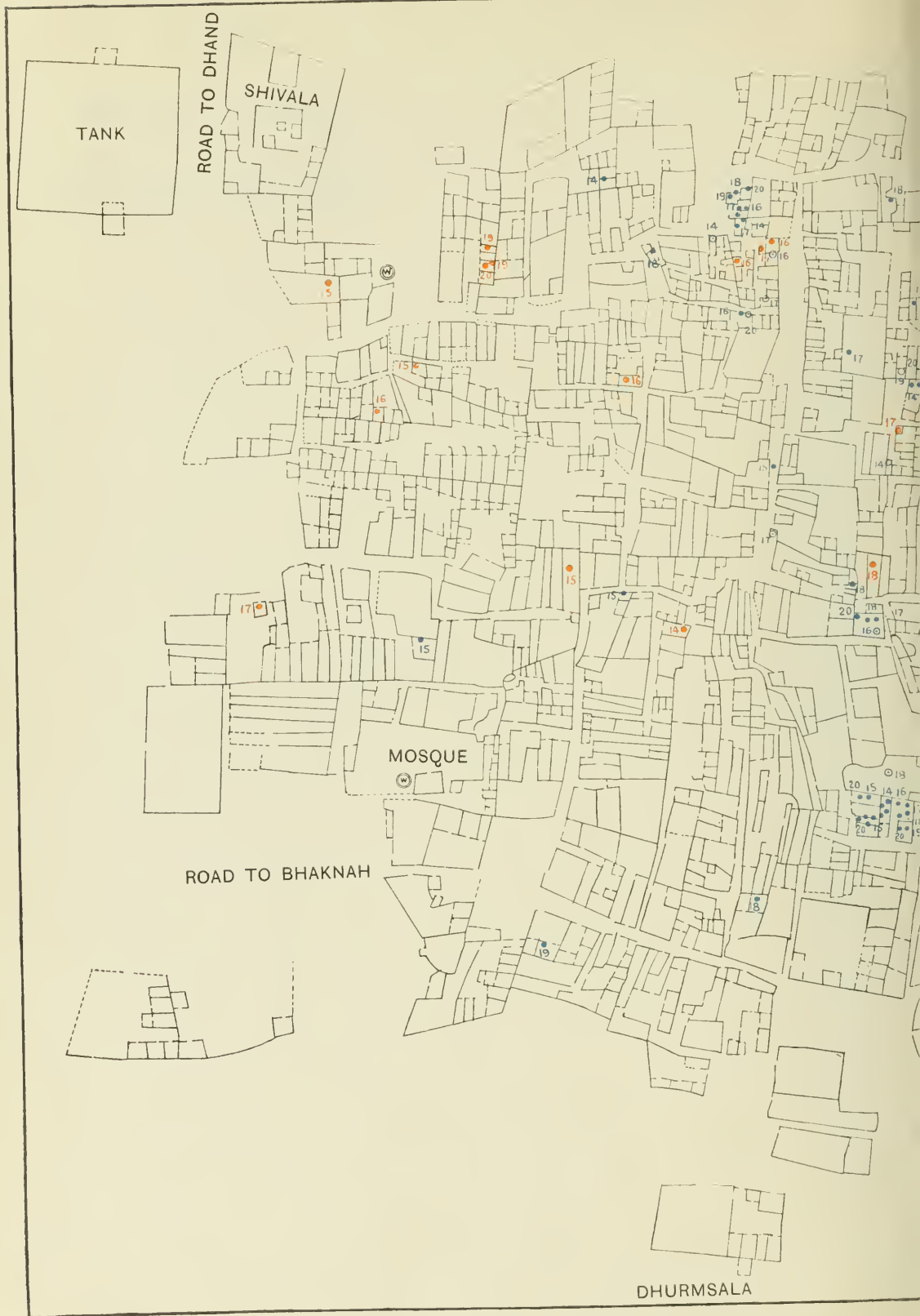


## MAP 9

### KASEL VILLAGE

Seventh week of epizootic,  
14 May, 1906 to 20 May, 1906



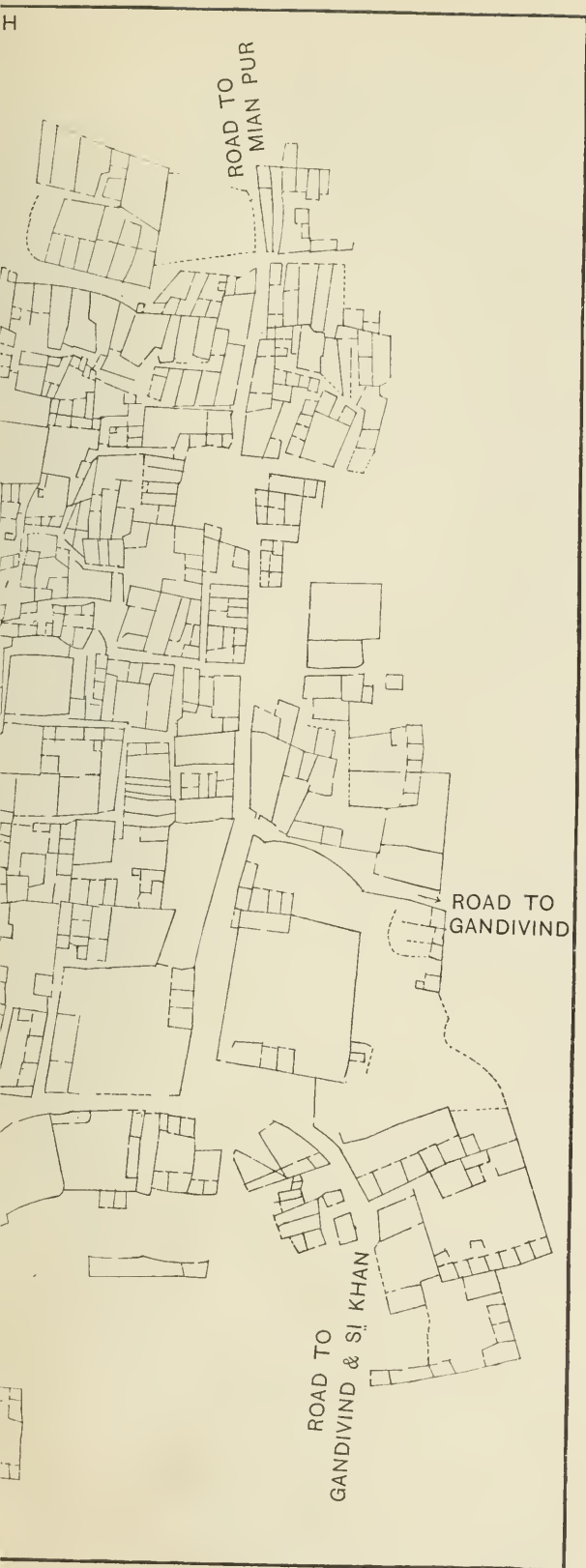


# KASEL VILLAGE

Seventh week of epizootic,  
14 May, 1906 to 20 May, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat

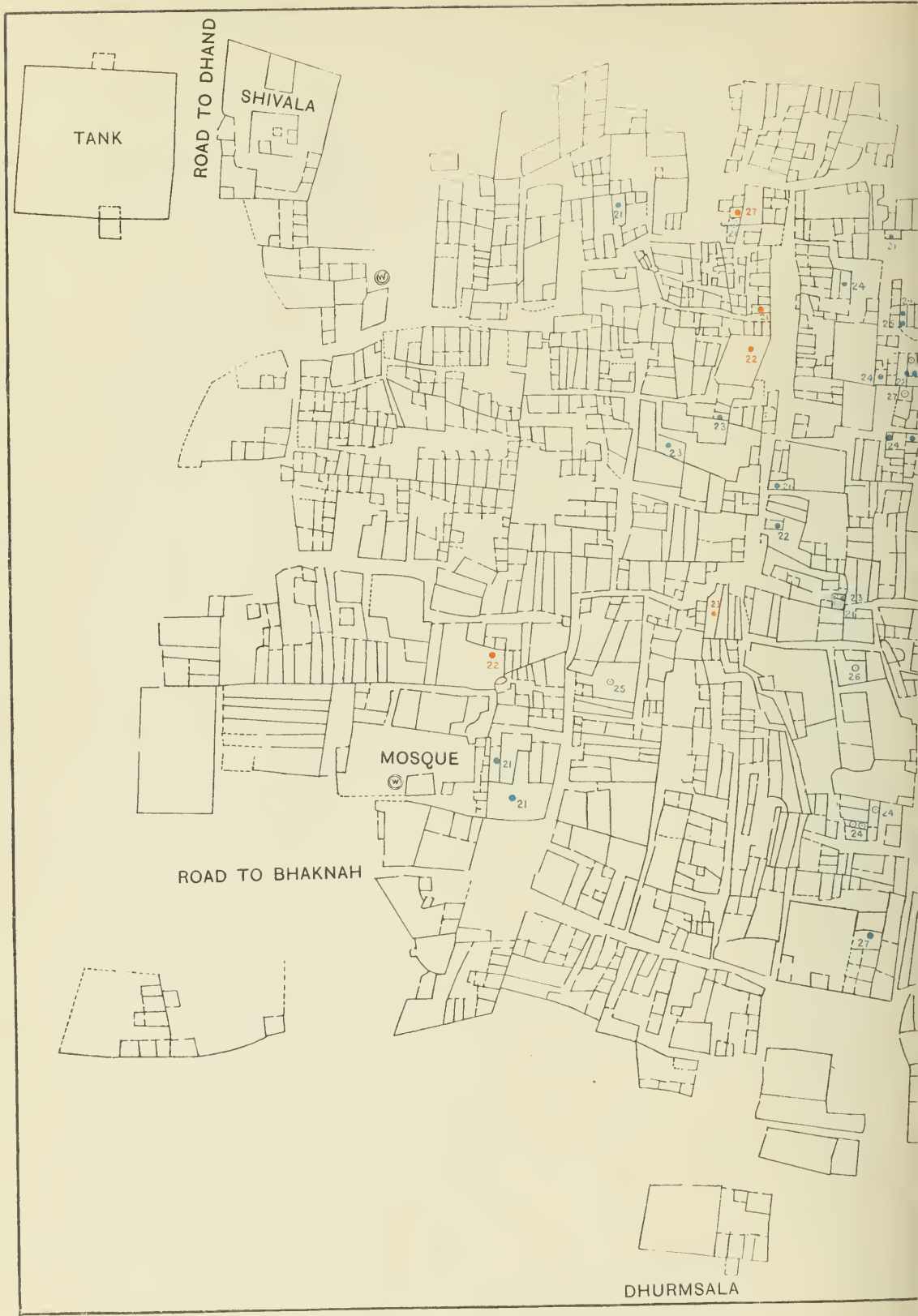




## MAP 10

### KASEL VILLAGE

Eighth week of epizootic,  
21 May, 1906 to 27 May, 1906



TANK

ROAD TO DHAND

SHIVALA

MOSQUE

ROAD TO BHAKNAH

DHURMSALA



# KASEL VILLAGE

Eighth week of epizootic,  
21 May, 1906 to 27 May, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat

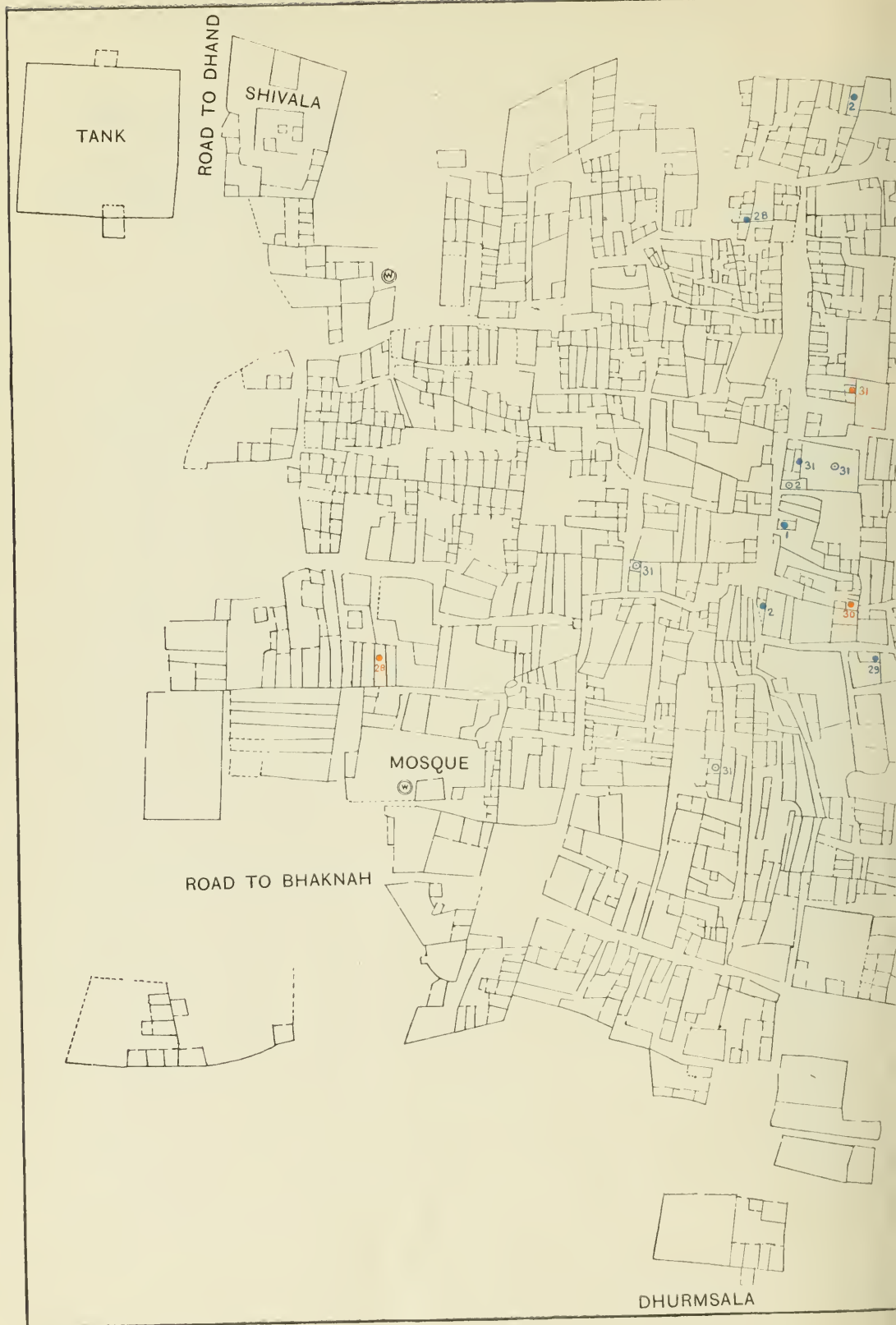




## MAP 11

### KASEL VILLAGE

Ninth week of epizootic,  
28 May, 1906 to 3 June, 1906



## KASEL VILLAGE

Ninth week of epizootic,  
28 May, 1906 to 3 June, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



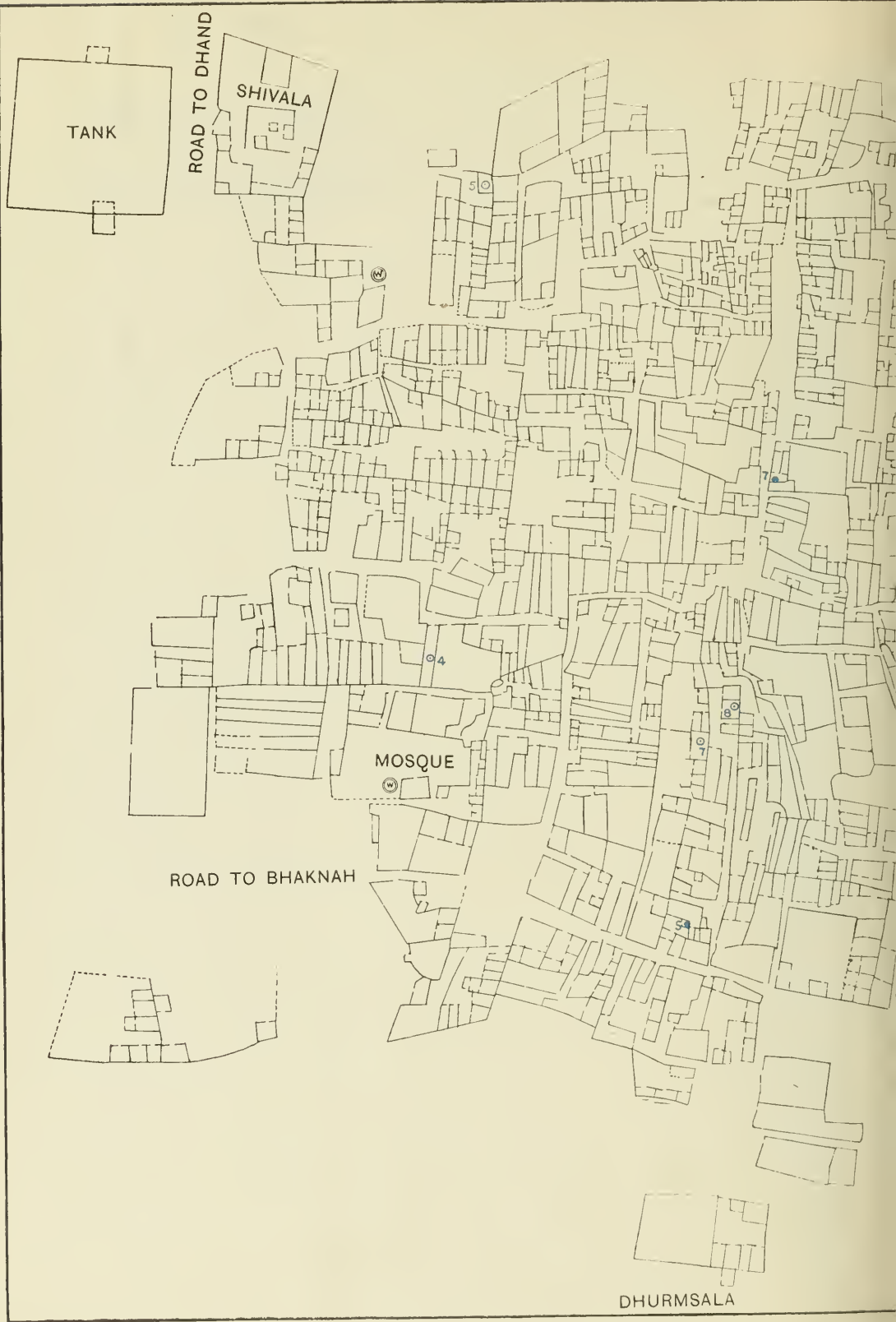




## MAP 12

### KASEL VILLAGE

Tenth week of epizootic,  
4 June, 1906 to 10 June, 1906

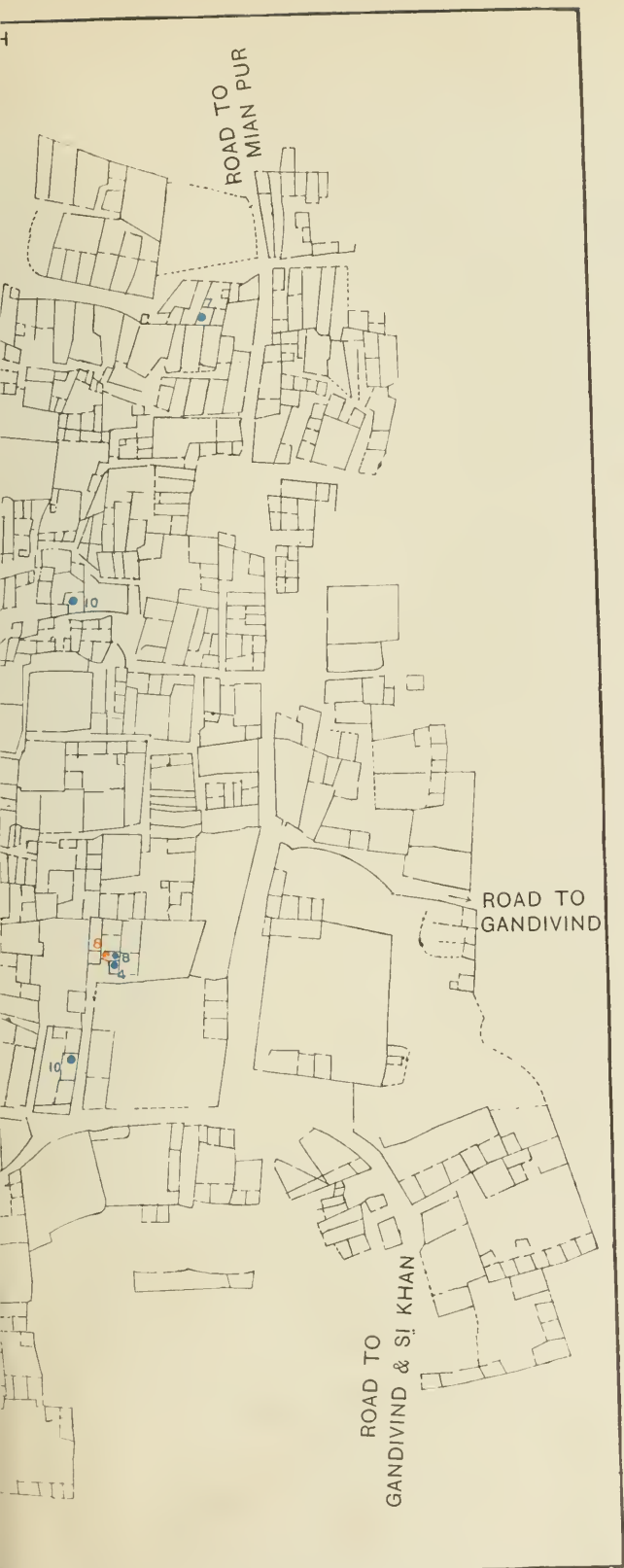


## KASEL VILLAGE

Tenth week of epizootic,  
4 June, 1906 to 10 June, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



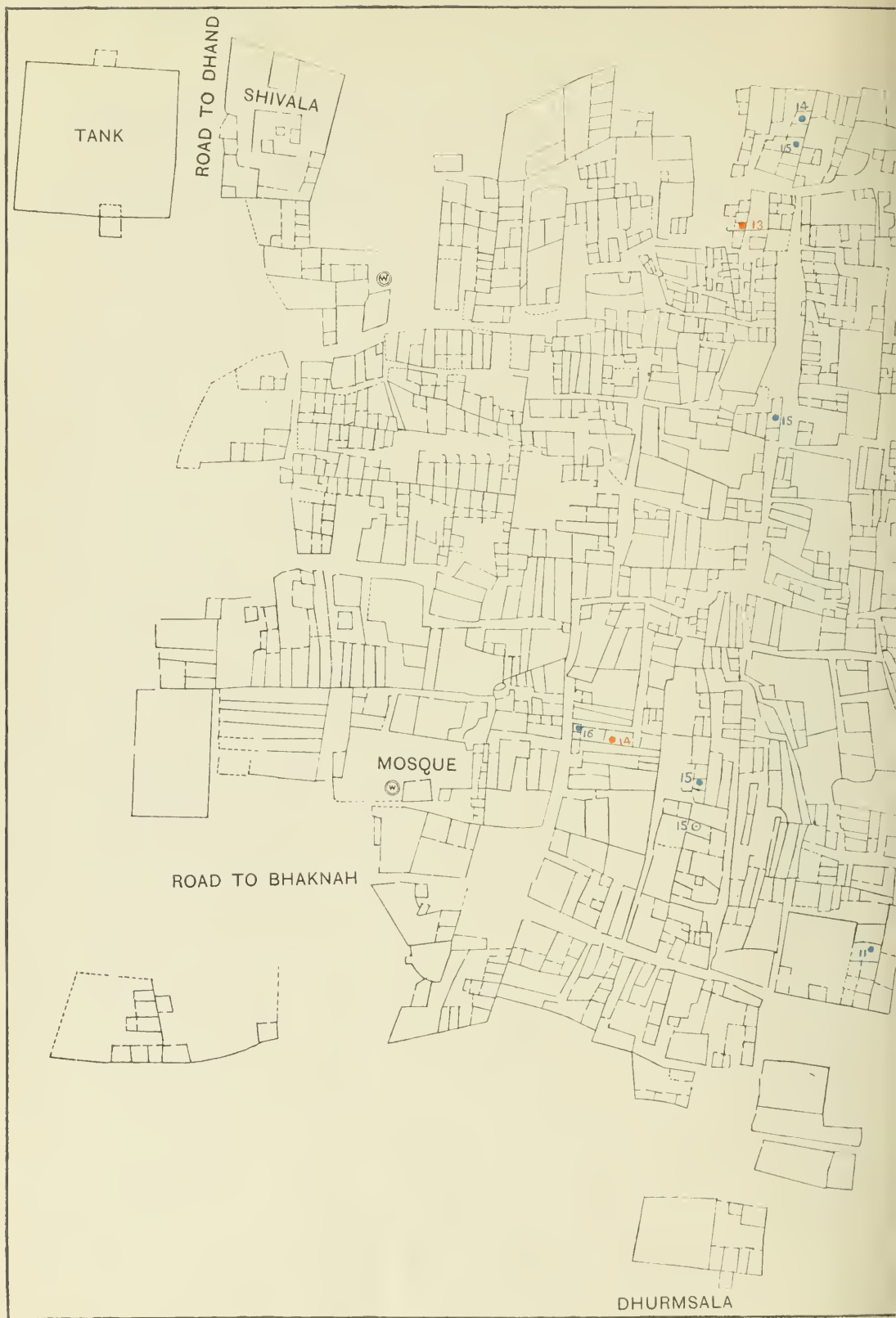




## MAP 13

### KASEL VILLAGE

Eleventh week of epizootic,  
11 June, 1906 to 17 June, 1906



# KASEL VILLAGE

Eleventh week of epizootic,  
11 June, 1906 to 17 June, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)
- Putrid or mummified rat



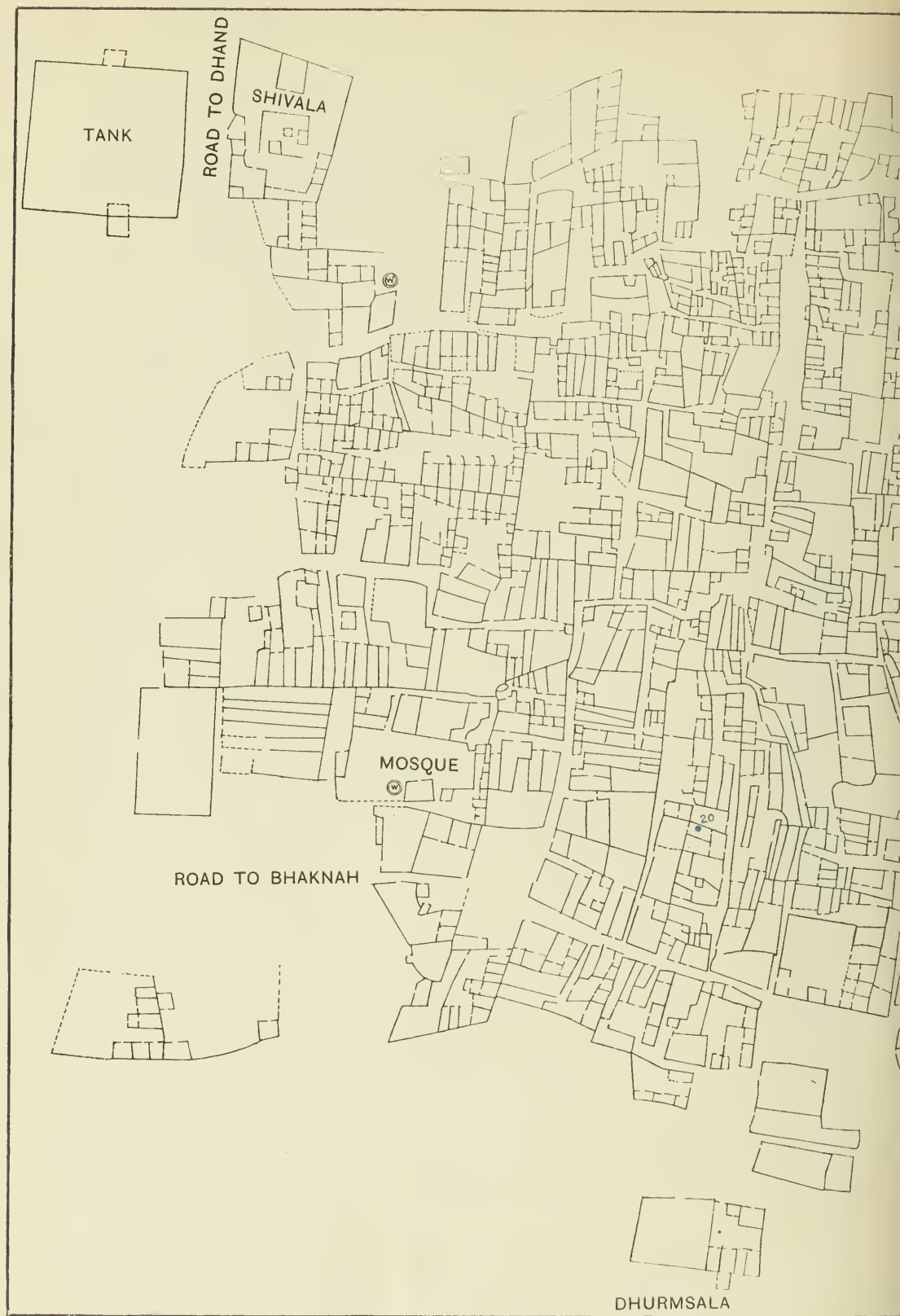


## MAP 14

### KASEL VILLAGE

Twelfth week of epizootic,  
18 June, 1906 to 24 June, 1906



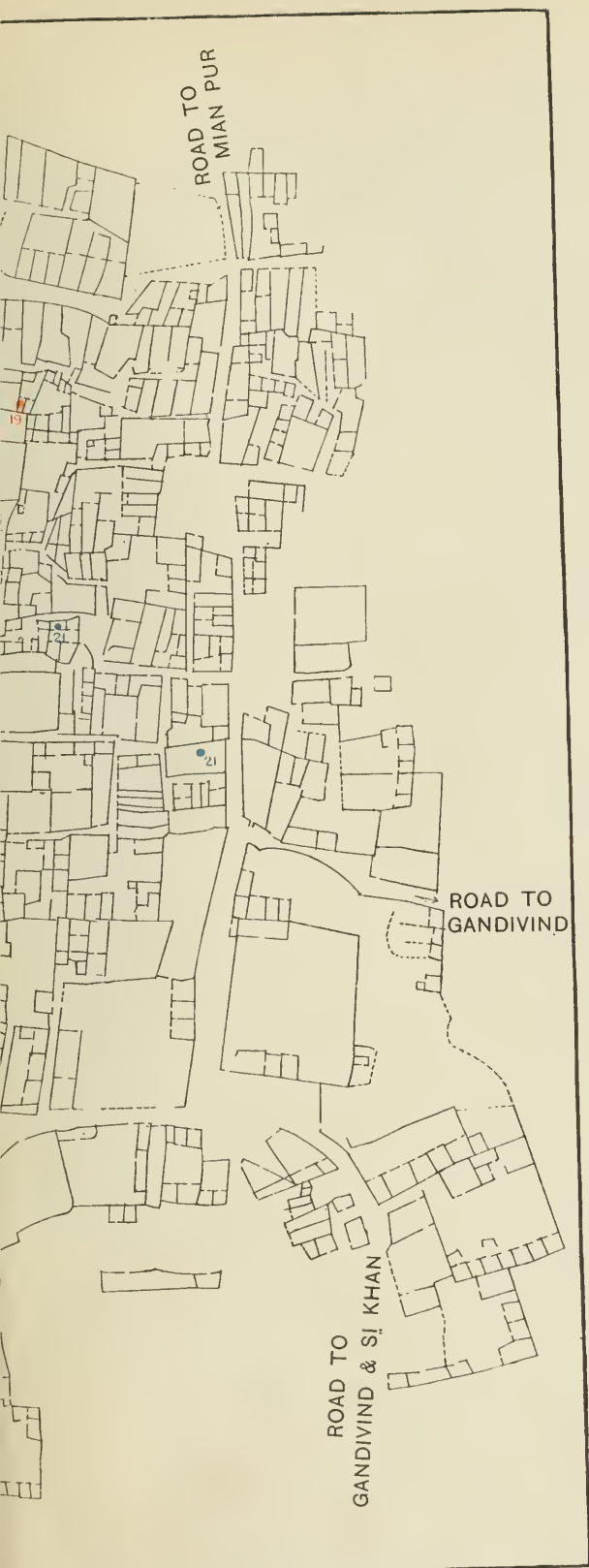


# KASEL VILLAGE

Twelfth week of epizootic,  
18 June, 1906 to 24 June, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)

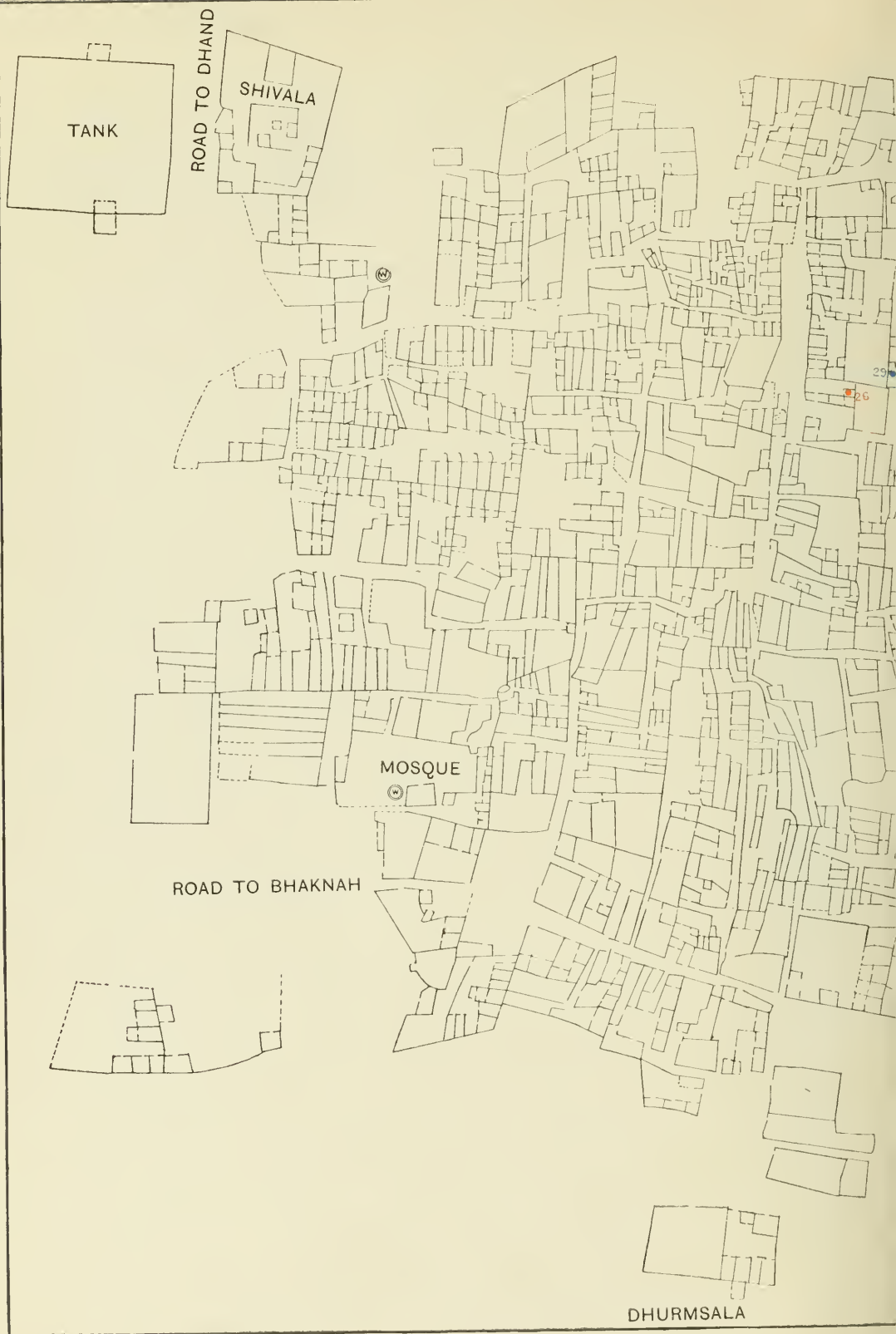




## MAP 15

### KASEL VILLAGE

Thirteenth week of epizootic,  
25 June, 1906 to 1 July, 1906



TANK

ROAD TO DHAND

SHIVALA

MOSQUE

ROAD TO BHAKNAH

DHURMSALA

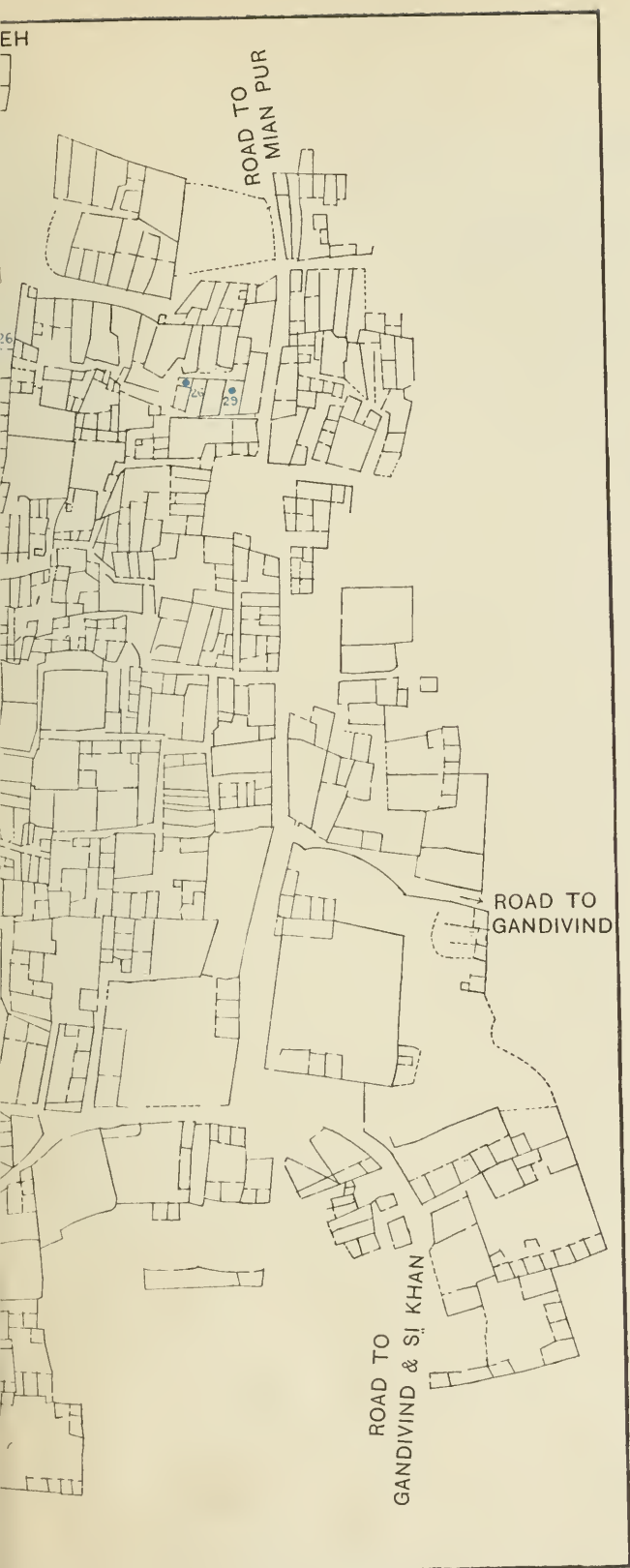


# KASEL VILLAGE

Thirteenth week of epizootic,  
25 June, 1906 to 1 July, 1906

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)

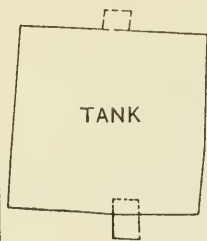




## MAP 16

### KASEL VILLAGE

Fourteenth and fifteenth weeks of  
epizootic, 2 July, 1906 to 17 July, 1906



ROAD TO DHAND

SHIVALA

MOSQUE

ROAD TO BHAKNAH

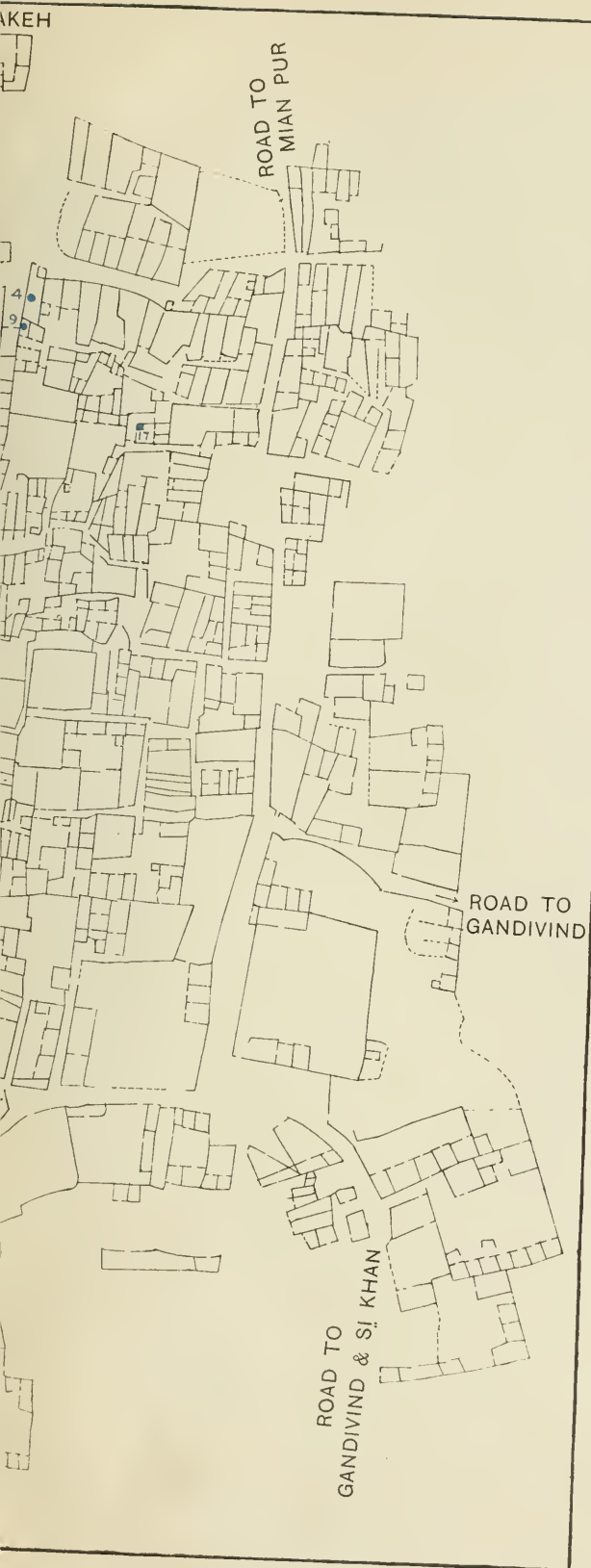
DHURMSALA

## KASEL VILLAGE

Fourteenth and fifteenth weeks  
epizootic, 2 July, 1906 to 17 July,

100 feet

- Human plague case (date of attack)
- Plague infected rat (date)



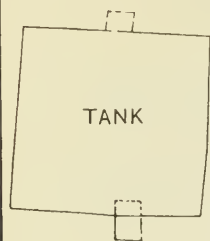




## MAP 17

### KASEL VILLAGE

Period after the epizootic,  
18 July, 1906 to 5 Dec. 1906



ROAD TO DHAND

SHIVALA

23

28 10

16 10

MOSQUE

24

ROAD TO BHAKNAH

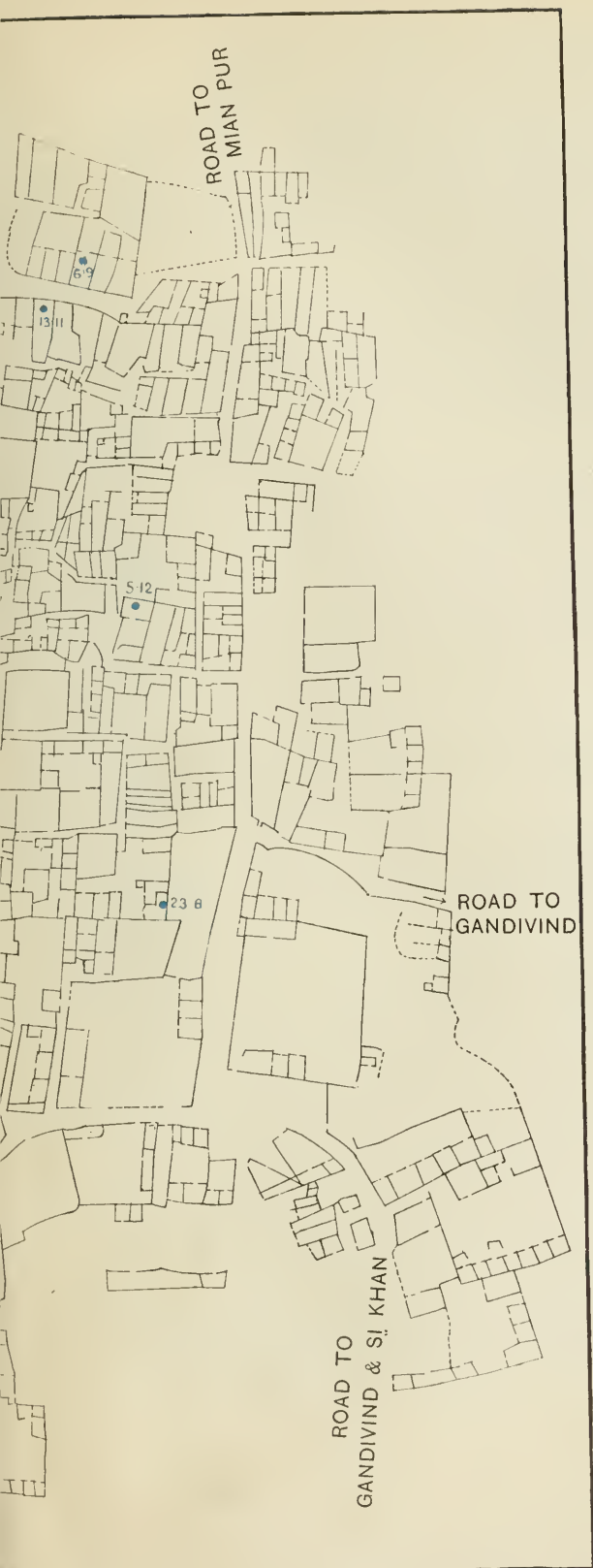
DHURMSALA

# KASEL VILLAGE

Period after the epizootic,  
18 July, 1906 to 5 Dec. 1906

100 feet

● Chronic plague rat (date)







were trapped, the first on the 23rd August and the last on the 5th December (*vide* Map 17). It will be noticed that all these chronic plague rats were taken in houses in which or in the neighbourhood of which acute plague rats had been found during the epizootic period.

#### 4. *Origin of the epizootic.*

In Kasel, as in Dhand, we were unable to come to a definite conclusion regarding the origin of the epizootic. At the time the epizootic began in Kasel several of the neighbouring villages, including Dhand, were infected. There was thus ample opportunity for the infection being brought in.

We have seen that the only imported case of plague, previous to the epizootic period, arrived in Kasel on the 12th March and died on the 14th (Appendix, Case 1). There are several reasons, however, for thinking that the disease among the rats did not originate from infection introduced by this case. Briefly stated these are as follows:

1. Guinea-pig experiments carried out on the 15th March in house No. 746 (the residence of Case No. 1), to determine whether the house was infective, gave negative results.

2. House No. 746 and all the houses in its vicinity were trapped on the 26th March and about 50 rats were taken. None of these rats was found to be plague-infected.

3. A reference to Map 1 will show that the houses where the first plague rats were found are at a considerable distance from house No. 746, and that no plague-infected rats were found in the immediate neighbourhood of the latter house, until several weeks after the epizootic had begun.

Having failed to satisfactorily connect the origin of the epizootic with the only imported case of plague, we made careful inquiries in all the houses in the neighbourhood where the first plague rats were found, in order to determine whether any healthy person who had been exposed to infection elsewhere had recently arrived there. The only facts that came to light suggesting that infection may have been introduced in this way were those in connection with the occupants of house No. 122, in which the first plague-infected rat was found on the 2nd April. House No. 122 is a goldsmith's shop, kept by two brothers who lived in house No. 177 Dhand. These men worked by day in their shop in Kasel, returning to Dhand at night. On the 6th of March a plague-infected rat had been found in the house occupied by their uncle, the next house to No. 177 Dhand. Subsequently a few plague rats were found in

TABLE XXV.

*Showing the places at which, and the first and last dates on which, plague-infected, putrid or mummified rats were taken. The entries are consecutively numbered in accordance with the dates on which the first rat was taken or found. References to the human cases are inserted in the series in their order.*

Case 1. Imported 12/3/06. House 746.							
Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
1	122	2/4/06	—	1	29/4/06	31/5/06	2
2	114	3/4/06	4/4/06	2	10/4/06	—	1
Case 2. Attacked 5/4/06. House 68							
3	119	6/4/06	9/4/06	2	—	—	—
4	121	6/4/06	—	1	—	—	—
5	896	7/4/06	—	1	—	—	—
6	894	11/4/06	—	1	—	—	—
7	36	13/4/06	—	1	—	—	—
8	842	14/4/06	—	1	—	—	—
9	902	14/4/06	18/4/06	2	—	—	—
10	961	15/4/06	—	1	—	—	—
Case 3. Attacked 15/4/06. House No. 1010							
11	249	16/4/06	—	1	—	—	—
12	82	17/4/06	1/5/06	3	—	—	—
13	50	17/4/06	—	1	—	—	—
14	55	17/4/06	—	1	—	—	—
15	376	16/5/06	18/5/06	4	17/4/06	—	1
16	869	17/4/06	—	1	—	—	—
Case 4. Attacked 17/4/06. House No. 894							
17	111	18/4/06	19/4/06	2	—	—	—
18	118	18/4/06	—	1	—	—	—
Case 5. Attacked 18/4/06. House No. 1158							
Case 6. Imported 18/4/06. House No. 828							
19	843	19/4/06	22/4/06	2	—	—	—
20	112	19/4/06	—	1	—	—	—
21	784	19/4/06	20/4/06	2	—	—	—
22	960	19/4/06	23/4/06	2	—	—	—
23	248	20/4/06	24/4/06	2	12/5/06	—	1
Case 7. Attacked 20/4/06. House 902							
24	113	—	—	—	21/4/06	—	1
25	782	21/4/06	29/4/06	2	28/4/06	—	1
26	881	22/4/06	27/4/06	2	—	—	—
27	779	22/4/06	26/4/06	3	—	—	—
28	969	23/4/06	—	1	—	—	—
29	833	—	—	—	24/4/06	—	1
30	12	24/4/06	—	1	—	—	—

# *Reports on Plague Investigations in India* 947

## Case 8. Attacked 24/4/06. House 901

Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
31	345	25/4/06	—	1	—	—	—
32	883	25/4/06	—	1	—	—	—
33	769	25/4/06	1/5/06	3	—	—	—
34	815	25/4/06	—	1	—	—	—

## Case 9. Attacked 25/4/06. House 901

## Case 10. Attacked 25/4/06. House 897

35	781	26/4/06	—	1	—	—	—
36	104	26/4/06	—	1	—	—	—
37	43	27/4/06	27/4/06	2	—	—	—
38	343	27/4/06	—	1	—	—	—
39	390	27/4/06	2/5/06	6	—	—	—

## Case 11. Attacked 27/4/06. House 857

40	837	—	—	—	28/4/06	—	1
41	135	—	—	—	28/4/06	—	1

## Case 12. Attacked 28/4/06. House 246

## Case 13. Attacked 28/4/06. House 838

42	268	29/4/06	—	1	—	—	—
43	827	—	—	—	29/4/06	29/4/06	2
44	96	29/4/06	—	1	—	—	—
45	391	29/4/06	2/5/06	4	—	—	—
46	911	29/4/06	—	1	—	—	—
47	853	29/4/06	—	1	—	—	—
48	823	30/4/06	3/5/06	2	—	—	—
49	97	—	—	—	30/4/06	—	1

## Case 14. Attacked 30/4/06. House No. 868

50	344	1/5/06	—	1	7/5/06	—	1
51	99	1/5/06	—	1	—	—	—
52	806	1/5/06	—	1	1/5/06	—	1
53	795	2/5/06	—	1	—	—	—
54	250	2/5/06	—	1	—	—	—
55	393	2/5/06	—	1	3/5/06	10/5/06	2
56	266	2/5/06	—	1	—	—	—
57	807	2/5/06	3/5/06	2	—	—	—
58	1013	2/5/06	—	1	—	—	—

## Case 15. Attacked 2/5/06. House No. 82

59	253 (Lane)	3/5/06	—	1	—	—	—
60	389	3/5/06	—	1	—	—	—
61	918	—	—	—	3/5/06	4/6/06	2

## Case 16. Attacked 3/5/06. House No. 81

62	51	4/5/06	7/5/06	6	6/5/06	8/5/06	3
63	757	4/5/06	8/5/06	2	—	—	—
64	759	—	—	—	4/5/06	—	1

*Plague in Kasel*

## Case 17. Attacked 4/5/06. House No. 1243

Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
65	424	5/5/06	—	1	—	—	—
66	255	5/5/06	9/5/06	5	6/5/06	12/5/06	5
67	1257	5/5/06	—	1	—	—	—
68	392	5/5/06	7/5/06	3	—	—	—
69	953	5/5/06	—	1	—	—	—
70	421	8/5/06	—	1	5/5/06	—	1
71	726	5/5/06	—	1	—	—	—
72	422	5/5/06	7/5/06	5	8/5/06	—	1

## Case 18. Attacked 5/5/06. House No. 83

73	339	6/5/06	—	1	—	—	—
74	259 (Lane)	—	—	—	6/5/06	6/5/06	2
75	218	6/5/06	—	1	—	—	—
76	340	—	—	—	6/5/06	—	1
77	776	—	—	—	6/5/06	—	1
78	411	—	—	—	7/5/06	11/5/06	2
79	233	7/5/06	—	1	—	—	—
80	101	—	—	—	7/5/06	—	1
81	763	7/5/06	—	1	—	—	—
82	93	—	—	—	7/5/06	—	1
83	32	7/5/06	—	1	—	—	—
84	796	7/5/06	—	1	—	—	—
85	790	7/5/06	9/5/06	2	—	—	—

## Case 19. Attacked 7/5/06. House No. 775

## Case 20. Attacked 7/5/06. House No. 43

86	758	7/5/06	—	1	—	—	—
87	935	8/5/06	—	1	—	—	—
88	980	8/5/06	12/5/06	3	11/5/06	—	1
89	41	8/5/06	—	1	—	—	—
90	760	—	—	—	8/5/06	—	1
91	259	—	—	—	8/5/06	25/5/06	2
92	63	—	—	—	8/5/06	—	1
93	745	8/5/06	28/5/06	5	21/5/06	—	1
94	102	8/5/06	—	1	9/5/06	9/5/06	2
95	981	8/5/06	11/5/06	3	9/5/06	—	1

## Case 21. Attacked 8/5/06. House No. 902

## Case 22. Attacked 8/5/06. House No. 78

## Case 23. Attacked 8/5/06. House No. 756

96	87	—	—	—	9/5/06	—	1
97	923	9/5/06	11/5/06	2	—	—	—
98	397	—	—	—	9/5/06	9/5/06	2
99	208	13/7/06	—	1	9/5/06	—	1
100	395	9/5/06	20/5/06	5	12/5/06	16/5/06	3
101	889	—	—	—	9/5/06	—	1

# *Reports on Plague Investigations in India*      949

Case 24. Attacked 9/5/06. House No. 135  
 Case 25. Attacked 9/5/06. House No. 881  
 Case 26. Attacked 9/5/06. House No. 757  
 Case 27. Attacked 9/5/06. House No. 776  
 Case 28. Attacked 9/5/06. House No. 8  
 Case 29. Attacked 9/5/06. House No. 391

Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
102	346	10/5/06	—	1	—	—	—
103	94	11/5/06	—	1	10/5/06	—	1
104	24	10/5/06	—	1	—	—	—
105	861	10/5/06	—	1	—	—	—
106	159	10/5/06	—	1	—	—	—
107	1023	10/5/06	—	1	—	—	—

Case 30. Attacked 10/5/06. House No. 759  
 Case 31. Attacked 10/5/06. House No. 486

108	341	11/5/06	—	1	—	—	—
109	33	11/5/06	—	1	—	—	—
110	927	11/5/06	—	1	—	—	—
111	743	11/5/06	20/5/06	4	—	—	—
113	752	11/5/06	—	1	—	—	—

Case 32. Attacked 11/5/06. House No. 391  
 Case 33. Attacked 11/5/06. House No. 43  
 Case 34. Attacked 11/5/06. House No. 256

114	216	12/5/06	—	1	—	—	—
115	244	12/5/06	—	1	—	—	—
116	217	—	—	—	12/5/06	—	1
117	235	—	—	—	12/5/06	—	1

Case 35. Attacked 12/5/06. House No. 422  
 Case 36. Attacked 12/5/06. House No. 767  
 Case 37. Attacked 12/5/06. House No. 225  
 Case 38. Attacked 12/5/06. House No. 133  
 Case 39. Attacked 12/5/06. House No. 134  
 Case 40. Attacked 12/5/06. House No. 818

118	8	13/5/06	—	1	—	—	—
119	240	13/5/06	2/6/06	2	—	—	—
120	181	13/5/06	14/5/06	3	27/5/06	—	1
121	920	13/5/06	15/5/06	2	—	—	—

Case 41. Attacked 13/5/06. House No. 391  
 Case 42. Attacked 13/5/06. House No. 158

122	203	—	—	—	14/5/06	—	1
123	746	14/5/06	17/5/06	2	—	—	—
124	375	14/5/06	14/5/06	4	24/5/06	—	1
125	767	—	—	—	14/5/06	—	1
126	1003	14/5/06	—	1	—	—	—



*Plague in Kasel*

Case 43. Attacked 14/5/06. House No. 263

Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
127	135 (Laue)	15/5/06	—	1	—	—	—
128	371	15/5/06	20/5/06	2	—	—	—
129	373	15/5/06	20/5/06	4	24/5/06	24/5/06	2
130	256	15/5/06	—	1	—	—	—
131	454	—	—	—	15/5/06	—	1

Case 44. Attacked 15/5/06. House No. 823

Case 45. Attacked 15/5/06. House No. 908

132	785	16/5/06	—	1	—	—	—
133	185	16/5/06	—	1	—	—	—
134	791	16/5/06	—	1	20/5/06	—	1
135	750	—	—	—	16/5/06	—	1
136	727	16/5/06	25/5/06	3	—	—	—

Case 46. Attacked 16/5/06. House No. 855

Case 47. Attacked 16/5/06. House No. 85

Case 48. Attacked 16/5/06. House No. 758

Case 49. Attacked 16/5/06. House No. 748

137	156	17/5/06	—	1	—	—	—
138	226	—	—	—	17/5/06	—	1
139	754	—	—	—	17/5/06	—	1

Case 50. Attacked 17/5/06. House No. 199

Case 51. Attacked 17/5/06. House No. 936

Case 52. Attacked 17/5/06. House No. 749

140	379	—	—	—	18/5/06	—	1
141	337	18/5/06	—	1	—	—	—
142	232	18/5/06	—	1	—	—	—
143	731	18/5/06	—	1	—	—	—

(Compound behind)

Case 53. Attacked 18/5/06. House No. 398

Case 54. Attacked 18/5/06. House No. 961

144	1283	19/5/06	—	1	—	—	—
145	714	—	—	—	19/5/06	—	1
146	377	19/5/06	20/5/06	2	—	—	—

Case 55. Attacked 19/5/06. House No. 979

Case 56. Attacked 19/5/06. House No. 981

147	712	20/5/06	22/5/06	3	25/5/06	—	—
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Case 57. Attacked 20/5/06. House No. 981

148	732	21/5/06	—	1	—	—	—
149	35	21/5/06	—	1	—	—	—
150	31	21/5/06	—	1	—	—	—
151	1015	21/5/06	—	1	—	—	—
152	232	23/5/06	—	1	21/5/06	21/5/06	3

# *Reports on Plague Investigations in India* 951

		Case 58. Attacked 21/5/06. House No. 754					
		Plague-infected rats			Mummified or putrid rats		
Serial No.	House No.	First	Last	Total	First	Last	Total
153	224	22/5/06	—	1	—	—	—
		Case 59. Attacked 22/5/06. House No. 102					
		Case 60. Attacked 22/5/06. House No. 672					
		Case 61. Attacked 22/5/06. House No. 917					
154	109	23/5/06	—	1	—	—	—
155	105	23/5/06	—	1	—	—	—
156	459	—	—	—	23/5/06	29/5/06	2
157	498	23/5/06	—	1	—	—	—
		Case 62. Attacked 23/5/06. House No. 244					
158	715	24/5/06	29/6/06	2	—	—	—
159	199	24/5/06	—	1	—	—	—
160	169	24/5/06	—	1	—	—	—
161	196	24/5/06	—	1	—	—	—
162	189	25/5/06	—	1	—	—	—
163	696	25/5/06	—	1	—	—	—
164	384	—	—	—	26/5/06	—	1
165	213	26/5/06	—	1	2/6/06	—	1
166	355	27/5/06	11/6/06	2	—	—	—
		Case 63. Attacked 27/5/06. House No. 744					
		Case 64. Attacked 28/5/06. House No. 923					
167	383	29/5/06	—	1	—	—	—
168	439	30/5/06	—	1	—	—	—
169	734	30/5/06	—	1	30/5/06	—	1
170	728	30/5/06	30/5/06	2	—	—	—
		Case 65. Attacked 30/5/06. House No. 230 A					
171	211	31/5/06	7/6/06	2	—	—	—
172	210	—	—	—	31/5/06	—	1
173	971	—	—	—	31/5/06	5/6/06	2
174	296	—	—	—	31/5/06	—	1
175	490	31/5/06	—	1	—	—	—
		Case 66. Attacked 31/5/06. House No. 175					
		Case 67. Attacked 31/5/06. House No. 207					
176	432	1/6/06	—	1	—	—	—
177	223	1/6/06	—	1	—	—	—
178	1042	2/6/06	—	1	—	—	—
179	1206	2/6/06	11/6/06	2	—	—	—
180	493	4/6/06	—	1	—	—	—
181	302	5/6/06	—	1	—	—	—
182	295	—	—	—	7/6/06	—	1
183	693	7/6/06	—	1	—	—	—
184	492	8/6/06	—	1	—	—	—
185	321	—	—	—	8/6/06	—	1

*Plague in Kasel*

Case 68. Attacked 8/6/06. House No. 492							
Serial No.	House No.	Plague-infected rats			Mummified or putrid rats		
		First	Last	Total	First	Last	Total
186	485	10/6/06	—	1	—	—	—
187	601	10/6/06	—	1	—	—	—
188	1162	—	—	—	11/6/06	—	1
Case 69. Attacked 11/6/06. House No. 1147							
Case 70. Attacked 13/6/06. House No. 745							
189	1034	14/6/06	—	1	—	—	—
190	444	14/6/06	—	1	—	—	—
Case 71. Attacked 14/6/06. House No. 270							
191	1036	15/6/06	—	1	—	—	—
192	138	15/6/06	—	1	—	—	—
193	615	15/6/06	—	1	—	—	—
194	297	15/6/06	—	1	—	—	—
195	316	20/6/06	—	1	15/6/06	—	1
196	686	16/6/06	29/6/06	2	—	—	—
197	272	16/6/06	—	1	—	—	—
Case 72. Attacked 19/6/06. House No. 662							
Case 73. Attacked 19/6/06. House No. 667							
198	552	21/6/06	—	1	—	—	—
199	508	21/6/06	—	1	—	—	—
200	700	23/6/06	26/6/06	2	—	—	—
201	682	26/6/06	—	1	—	—	—
Case 74. Attacked 26/6/06. House No. 175							
202	698	4/7/06	—	1	—	—	—
Case 75. Attacked 7/7/06. House No. 620							
203	669	9/7/06	—	1	—	—	—
204	643	17/7/06	—	1	—	—	—

houses in the same neighbourhood. Further, as we have already mentioned, about a week before the plague rat was found in their shop in Kasel, they had noticed a bad smell which they thought was due to a dead rat. This fact would suggest that the rats in this shop were actually the first in Kasel to become infected. It is quite possible, therefore, that the infection was brought from Dhand by the occupants of No. 122.

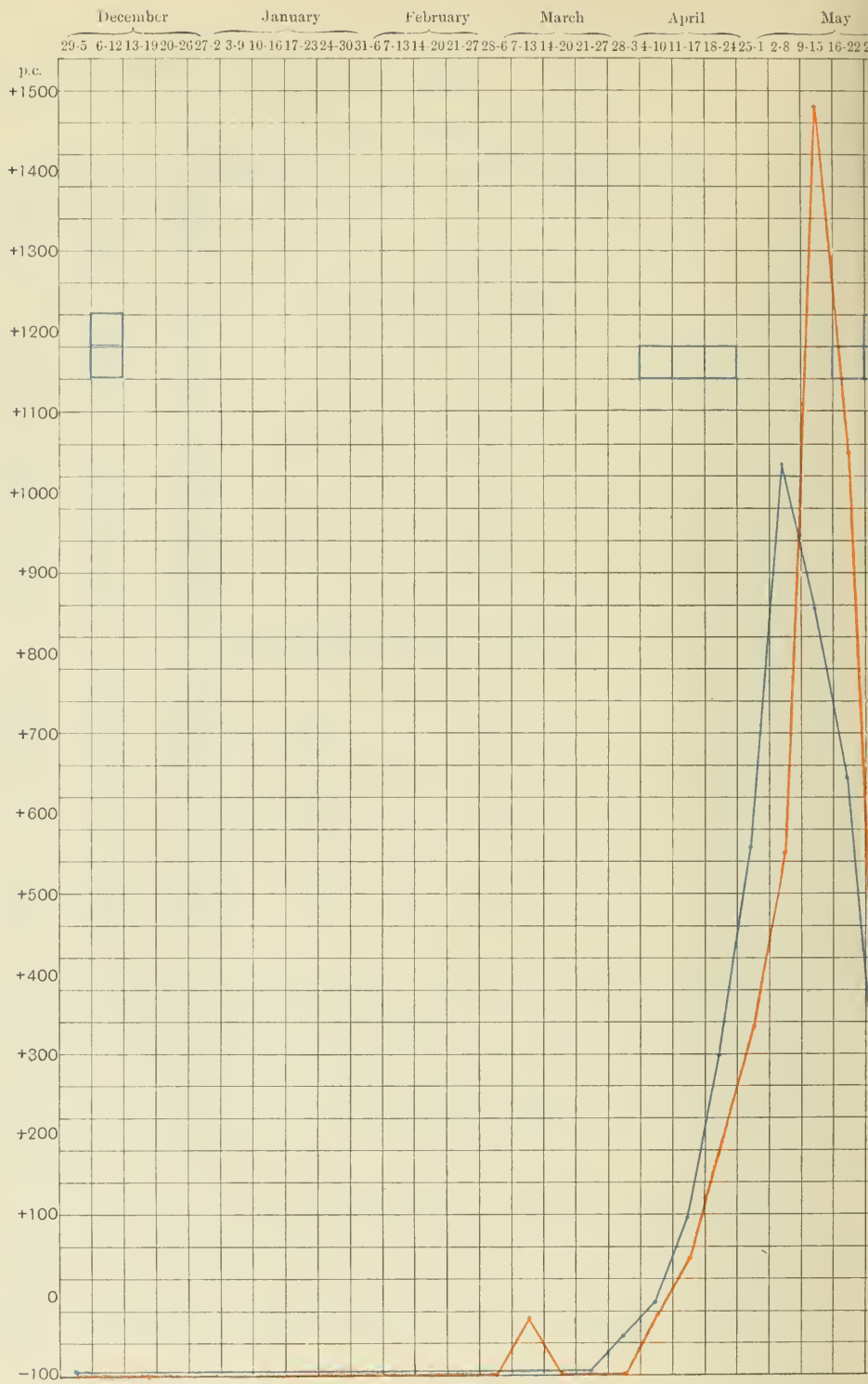
We have little to say as to the possibility of infection having been introduced into Kasel by infected rats which had migrated from another village. It is conceivable that such migration may have taken place from Dhand, which was the only infected village within three miles of Kasel. Had infection been introduced in this way we should, however,

PUNJAB IV

KASEL—PUNJAB

December, 1905 to November, 1906

1  
952



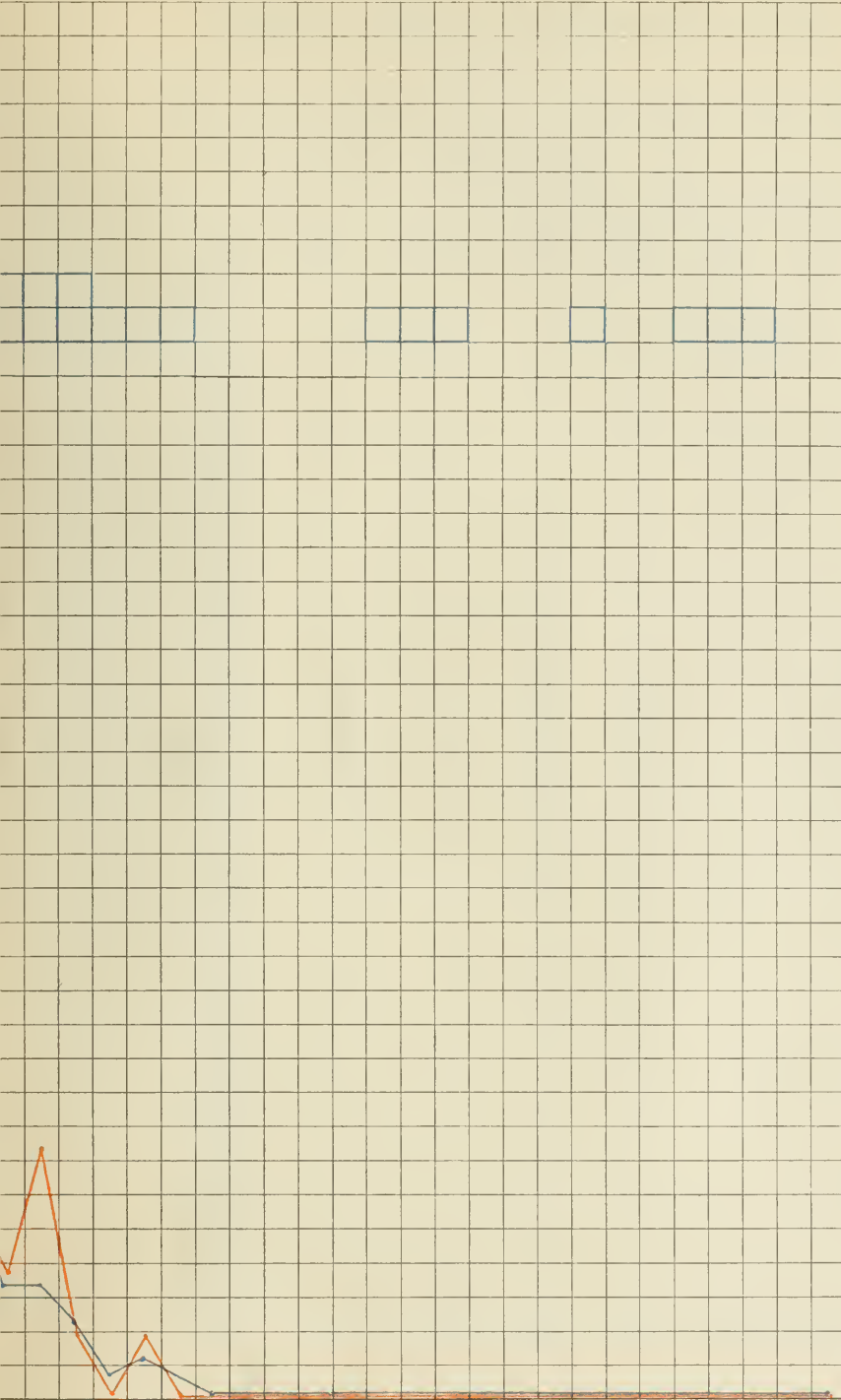
KASEL—

December, 1905 to

- Plague infected rats (acute)
- Chronic plague rats. One
- Human plague attacks, p.c.



June July August September October November  
 12 13 19 20 26 27 3 4 10 11 17 18 24 25 31 1 7 8 14 15 21 22 28 29 4 5 11 12 18 19 25 26 2 3 9 10 16 17 23 24 30 31 6 7 13 14 20 21 27



PUNJAB

September, 1906

above and below the mean

one rat

and below the mean

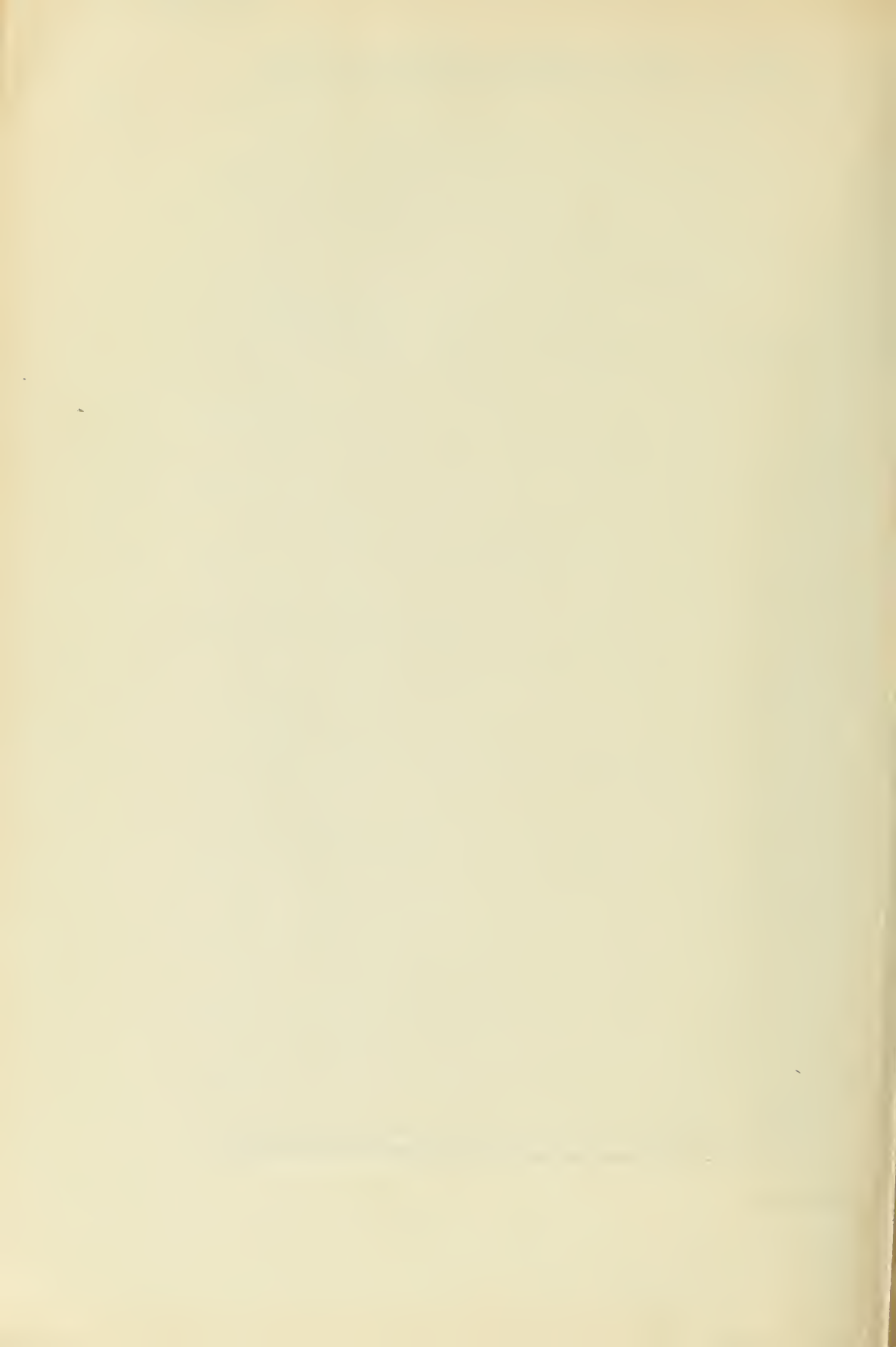


TABLE XXVI.

*Showing number of plague cases and plague-infected rats in Kasel week by week.*

Weekly period ending	Plague cases	Plague deaths	Rats			Grand Total
			Acute plague	Too putrid for diagnosis	Chronic plague	
5/12/05	—	—	—	—	—	—
12/12/05	—	—	—	—	2	2
19/12/05	—	—	—	—	—	—
26/12/05	—	—	—	—	—	—
2/1/06	—	—	—	—	—	—
9/1/06	—	—	—	—	—	—
16/1/06	—	—	—	—	—	—
23/1/06	—	—	—	—	—	—
30/1/06	—	—	—	—	—	—
6/2/06	—	—	—	—	—	—
13/2/06	—	—	—	—	—	—
20/2/06	—	—	—	—	—	—
27/2/06	—	—	—	—	—	—
6/3/06	—	—	—	—	—	—
13/3/06	1	—	—	—	—	—
20/3/06	—	1	—	—	—	—
27/3/06	—	—	—	—	—	—
3/4/06	—	—	2	—	—	2
10/4/06	1	—	4	1	1	6
17/4/06	2	1	9	1	1	11
24/4/06	4	2	18	2	1	21
1/5/06	6	4	30	8	—	38
8/5/06	9	5	52	23	—	75
15/5/06	22	10	44	22	—	66
22/5/06	16	7	34	11	1	46
29/5/06	3	6	14	9	2	25
5/6/06	3	2	11	8	2	21
12/6/06	2	1	6	3	1	10
19/6/06	4	1	6	1	2	9
26/6/06	1	1	4	—	2	6
3/7/06	—	—	1	—	1	2
10/7/06	1	—	2	—	—	2
17/7/06	—	—	1	—	1	2
24/7/06	—	—	—	—	—	—
31/7/06	—	—	—	—	—	—
7/8/06	—	—	—	—	—	—
14/8/06	—	—	—	—	—	—
21/8/06	—	—	—	—	—	—
28/8/06	—	—	—	—	1	1
4/9/06	—	—	—	—	—	—
11/9/06	—	—	—	—	1	1
18/9/06	—	—	—	—	—	—
25/9/06	—	—	—	—	—	—
2/10/06	—	—	—	—	—	—
9/10/06	—	—	—	—	1	1
16/10/06	—	—	—	—	—	—
23/10/06	—	—	—	—	—	—
30/10/06	—	—	—	—	1	1
6/11/06	—	—	—	—	1	1
13/11/06	—	—	—	—	1	1
20/11/06	—	—	—	—	—	—
27/11/06	—	—	—	—	—	—
Total	75	41	238	89	23	350

have expected that the epizootic would have begun on the outskirts of the village nearest to Dhand, instead of in the middle of the village.

Finally, with regard to the question of the origin of the epizootic by the lighting up of chronic rat plague into acute plague we need only point out that during the period before the epizootic no chronic plague rat had been taken in Kasel after the 12th December, 1905, namely, four months before the first acute plague rat was found. But it is important to note that the rats were not examined from 1st January to 20th February.

## V. RELATION BETWEEN THE EPIZOOTIC AND EPIDEMIC.

### 1. *Relation in time.*

The relation in time between the epizootic and epidemic becomes apparent by reference to Tables XXV and XXVI, and Chart 4. The latter shows the number of infected rats and the number of plague attacks for each week of the period during which observations were carried out in Kasel.

It will be seen from Table XXV, that the first acute plague rat was found on the 2nd April and the last on the 17th July, while the first indigenous human case was attacked on the 5th April and the last on the 6th July. During the remainder of the year the village was under observation no plague case occurred and no acute plague rat was found.

It will be seen from Chart 4 that the curves of rat plague and of human plague exhibit a very close correspondence, the variations of the curve of human plague following those of the rat plague curve at an interval of about a week.

It is evident then that in Kasel:

- (1) Rat plague preceded human plague<sup>1</sup>.
- (2) Human plague ceased shortly before the cessation of acute plague among the rats.
- (3) A quantitative relation existed between rat plague and human plague.

### 2. *Relation in place.*

This relation will be seen by reference to Maps 1 and also 3—16 and to Table XXV. Considered with reference to their association with plague rats the 75 plague cases may be grouped as follows:—

<sup>1</sup> The slight rise in the human plague attack curve before the rise in the epizootic curve was due to an imported case, namely, case 1.

*Group A. Imported Cases.*

I. *Cases suffering from plague when they arrived in Kasel.* The cases included in this group are two, namely, Nos. 1 and 6 (*vide* Appendix).

II. *Cases which were not attacked until after their arrival in Kasel, but whose infection was traced to sources outside the village.*

In this group are included two cases, namely, Nos. 5 and 11 (*vide* Appendix).

*Group B. Indigenous Cases.*

III. *Cases preceded by the finding of plague-infected rats in their actual residence.*

This group includes 25 cases, which occupied 19 houses. The cases are—Nos. 4, 7, 12, 15, 16, 20, 21, 25, 26, 29, 32, 33, 35, 41, 44, 48, 55, 56, 57, 59, 60, 63, 64, 68, and 70 (*vide* Appendix).

Further particulars regarding this group will be found in Table No. 28, to which we shall have occasion to refer later on.

IV. *Cases preceded by the finding of plague rats in the immediate neighbourhood, but not in the actual residence.*

This group includes 36 cases, namely, Nos. 8, 9, 10, 13, 14, 18, 19, 22, 23, 24, 27, 28, 30, 34, 36, 37, 38, 39, 40, 42, 43, 45, 46, 47, 49, 50, 51, 52, 53, 54, 58, 62, 65, 66, 74, and 75 (*vide* Appendix).

The degree of propinquity indicated by the expression “in the immediate neighbourhood” varies, and its value for any individual case must be determined by reference to the Appendix and Maps 1 and 3—16. That the association of the majority of the cases of this group with plague rats was, however, very close, will be apparent from the following additional details:—

(a) Plague rats were found in the houses immediately adjoining, and structurally one with, the residences of 13 cases, namely, Nos. 8, 9, 14, 18, 22, 23, 28, 30, 37, 42, 43, 65, and 66.

(b) Rats which were too putrid for diagnosis, in association with plague rats in the vicinity, were found in the actual residences of cases Nos. 24, 27, 30, 36, and 58, and in the houses immediately adjoining the residences of cases Nos. 13, 19, 38, 39, 47, 49, and 53.

(c) Plague rats were found in the residences of cases Nos. 28, 34, and 40, 4, 4, and 7 days, respectively, after attack. In 3 cases of this group, namely, Nos. 42, 54, and 62 (*vide* Appendix), it is difficult to correctly adjudge the place of infection.

*Case 42.* This case may have been infected either at his residence or at shop No. 255 which he frequented.



*Case 54.* We cannot suppose that the source of infection of this case was the plague rat found at her residence more than a month previous to her attack; on the contrary, the facts suggest that she owed her infection directly or indirectly to house No. 981.

*Case 62.* While the history of this case suggests that she was infected at Kasel, we cannot exclude the possibility of her having been infected at Hoshiarnagar.

*V. Cases not preceded by the finding of plague rats, either at their residence or in the immediate neighbourhood thereof.* Ten cases are included in this group, namely, Nos. 2, 3, 17, 31, 61, 67, 69, 71, 72, and 73.

These cases may be conveniently subdivided into:—

(1) Cases which had visited at infected houses prior to being attacked, and

(2) Cases in which the source of infection could not be definitely determined.

Sub-group (1) includes 6 cases, namely, Nos. 2, 3, 17, 31, 67, and 69.

The probable place of infection for each of these cases was as follows (*vide* Appendix):—

*Case 2.* House No. 121, where she visited, though infection at residence cannot be excluded.

*Case 3.* House No. 121, where she worked.

*Case 17.* House No. 249, where she worked.

*Case 31.* House No. 422, where she frequently visited.

*Case 67.* House No. 734, where she had stayed just before attack.

*Case 69.* House No. 492, where she visited.

Sub-group (2) includes 4 cases, namely, Nos. 61, 71, 72, and 73. Of these cases No. 61 was a doubtful case of plague. It is further to be noted that plague rats were found in the vicinity of the residences of the last three of these cases shortly after their attack.

We may summarise the data as regards the relation of the epizootic to the epidemic in place as follows:—

(1) Of 75 cases—4 were imported.

(2) Of the 71 indigenous cases 61 occurred in houses, in which or in the immediate vicinity of which plague-infected rats had been found prior to attack.

(3) Plague rats were not found in or near the residences of the remaining 10 cases prior to their attacks, but six of these cases had visited at houses where plague rats had been found.

(4) Of the remaining four cases one was a doubtful case, and plague

rats were found in the vicinity of the houses of the three other cases some days after they fell ill.

It will be apparent from what has been said above that we were able to trace a more intimate association between plague cases and plague rats in Kasel than we had succeeded in doing in Dhand. There is no doubt that this difference in the results obtained in the two villages can be, in part, explained by the fact that information of rats having died was obtained more readily in Kasel than in Dhand, as the Kasel people had ceased to look on us with suspicion at the time the epizootic began in their village.

TABLE XXVII.

*Showing particulars of plague cases preceded by plague rats in the same house.*

A. Plague cases occurring in houses in which dead plague rats were found only once.

House No.	Date of finding plague rats	Serial No. of cases	Dates of attack of cases	Interval between finding plague rats and cases
894	11/4/06	4	17/4/06	6 days
43	27/4/06 (2)	20 & 33	7/5/06 & 11/5/06	10 "
758	7/5/06	48	16/5/06	9 "
102	8/5/06	59	22/5/06	14 "
244	12/5/06	60	23/5/06	11 "

B. Plague cases which occurred in houses in which dead plague rats were found on more than one occasion.

House No.	Dates of finding plague rats	Serial No. of cases	Dates of attack of cases	Interval	
				between 1st plague rat and 1st case	between last plague rat and 1st case
902	14/4/06 & 18/4/06	7 & 21	20/4/06 & 8/5/06	6 days	2 days
246	20/4/06 & 24/4/06	12	28/4/06	8 "	4 "
81-82	23/4/06 & 1/5/06	15 & 16	2/5/06 & 3/5/06	9 "	1 day
881	22/4/06 & 27/4/06	25	9/5/06	17 "	12 days
757	4/5/06 & 8/5/06	26	9/5/06	5 "	1 day
391	29/4/06 & 2/5/06	29, 32 & 41	9/5/06, 11/5/06 & 13/5/06	10 "	7 days
422	5/5/06 & 7/5/06	35	12/5/06	7 "	5 "
823	30/4/06 & 3/5/06	44	15/5/06	15 "	12 "
979-980	8/5/06, 10/5/06 & 12/5/06	55	19/5/06	11 "	7 "
981	8/5/06, 9/5/06 & 11/5/06	56 & 57	19/5/06 & 20/5/06	11 "	8 "
744	11/5/06, 18/5/06, 19/5/06 & 20/5/06	63	27/5/06	16 "	7 "
923	9/5/06 & 11/5/06	64	28/5/06	19 "	17 "
492	4/6/06 & 8/6/06	68	8/6/06	4 "	—
745	16/5/06, 17/5/06 & 28/5/06	70	13/6/06	28 "	16 "

3. *Interval between the finding of dead plague rats in houses and the occurrence of the first plague case in them.*

Table XXVII shows that in the case of five houses, in which plague rats were found on only one occasion prior to attack, this interval varied from 6 to 14 days—the average interval being 10 days.

In 14 houses in which plague rats were found on more than one occasion prior to attack, the interval between the finding of the first plague rat and the first case varied from 5 to 28 days—the average being 12 days—while the interval between the last plague rat and the first case varied from 1 to 17 days, the average being 7 days.

It will be seen that these intervals correspond fairly closely with those deduced from the curves representing the *rattus* epizootic and the epidemic in Bombay. The explanation of this interval on the basis of the flea theory has already been discussed.

4. *Finding of plague-infected rats in houses not always followed by plague cases.*

The finding of plague rats in houses was not often followed by plague cases among the occupants. Thus, out of 86 occupied houses in which plague rats were found and in which evacuation was not carried out, only 15 or 17% furnished cases. The risk of infection of the occupants appeared to increase with the number of plague rats found. Thus out of 80 houses in which single plague rats were found only 3, or 4% of the whole, furnished cases, whereas plague cases among the occupants followed in 16 out of 57, or 28%, of the houses in which more than one plague rat was found.

The above figures refer to dead plague rats only.

No plague case among the occupants occurred in any of 18 occupied houses in which live plague rats only were taken.

5. *The influence of evacuation of houses, in which dead plague rats were found, on the incidence of plague on the occupants.*

Evacuation as carried out in Kasel was either partial or complete.

Partial evacuation consisted in the occupants removing into the courtyard adjoining their house. In such cases communication with the house was not always completely cut off, as frequently one or more of the family went in and out for grain and other necessities. Complete evacuation consisted in the occupants removing into another house and taking with them grain and other necessities. Their

new residence was often at a distance from the evacuated house, with which they had, as a rule, no communication.

Of 137 occupied houses in which plague rats were found, 51 were evacuated within a day or two of the finding of the first plague rat. Of these about half were completely evacuated. In the remaining 86 houses the occupants did not turn out. The occupants of the 51 evacuated houses numbered 266, of which 27 had been recently inoculated. Of the remaining 239 susceptible persons 5, or 2%, contracted plague. The occupants of the 86 houses, who did not turn out, numbered 418, of whom 14 were inoculated. Of the remaining 404 susceptible persons 20, or 5%, contracted plague.

Although these figures are small they appear to warrant the conclusion that evacuation of houses soon after the finding of plague rats in them appreciably reduces the incidence of plague among the occupants.

## APPENDIX.

### ABSTRACT OF PLAGUE CASES IN KASEL.

#### *Case 1.*

Harnamoon, male, aet. 3, Chuhra. Residence—746, Kasel. Left Kasel with his parents about 1st March and went to Attari and later to a village named Narli in Lahore district. At Attari the family attended the mourning party of a Chuhra who is said to have died of fever. They went on to Narli about 3rd or 4th March. Narli was apparently infected with plague at the time they visited it. Harnamoon is said to have got fever about 9th March. He was brought back to house No. 746, Kasel on 12th March. When examined on 13th March he had high fever (105° F.) and axillary and cervical buboes. He died on 14th March. Material taken from bubo on 13th March—films and cultures positive.

*Rats*—No plague rats (excluding two chronic plague taken in December 1905) had been found in the village previous to this case.

Guinea-pig experiment—negative.

Connected cases—nil.

#### *Case 2.*

Hukamkour, female, aet. 35, Jat Sikh. Residence—68, Kasel. Had not left the village for some months. Frequently visited at house No. 121. The day before her attack she had visited a family of Nais in house No. 73. This family had just moved into No. 73 from No. 114, where plague-infected rats had been found the previous day. Attack—5th April; fever and right inguinal bubo; convalescent 10th April, bubo subsided without suppuration.

*Rats*—None in the house or immediate vicinity; but several plague rats in the neighbourhood of house No. 121 where she used to visit.

Guinea-pig experiments—A guinea-pig kept for night of 6th April in patient's house died of plague. No fleas were recovered from this pig.

Connected cases—nil.

*Case 3.*

Isso, female, aet. 10, Mehra (Hindu, water-carrier). Residence—1010, Kasel. Had not left the village. Previous to her attack used frequently to take water to house No. 121, as well as to other houses. Attack—15th April; fever, right femoral and inguinal buboes. Died during night of 17/4/06.

*Rats*—None at residence or in its vicinity prior to attack. Plague rats in the neighbourhood of house No. 121, where she worked (*vide supra*).

Guinea-pig experiments—negative.

Connected cases—nil.

*Case 4.*

Jiwan, female, aet. 60, Kamiar. Residence—894, Kasel. Attack—17th April; fever, right inguinal bubo first noticed on 19th April. Convalescent, 27th April; bubo subsided without suppuration.

*Rats*—A dead plague-infected rat found at residence on 11th April.

Connected cases—nil.

*Case 5.*

Lachmi, female, aet. 20, Sansi (a beggar tribe). Residence—1158, Kasel. Is said to have gone to a village, named Wadhala, in Amritsar Tahsil on or about 10th April to see her brother who was ill with plague there. Wadhala village was reported infected early in April. She returned to Kasel on or about 12th April. Attack—about 18th April, fever and right inguinal bubo. Examined on 20th April, temperature 105° F. Patient died on 26th April.

*Rats*—No plague rats found in house or anywhere near.

Guinea-pig experiments—negative.

Connected cases—nil.

*Case 6.*

Bhani, female, aet. 80, Mahomedan Kamboh. Residence—828, Kasel. Patient went to visit Majitha, a town in Amritsar Tahsil, about 12th April to visit her daughter, who was reported to be ill with plague there. Her daughter died the day after her arrival. Bhani was attacked with fever in Majitha about 18th April. She was brought back to Kasel by her son (Case 11) on 22nd April. When examined on 24th, for the first time, she was moribund. Her relatives gave a history of fever, cough and slight expectoration. No bubo was found on examination.

*Rats*—No plague rats at residence. The nearest plague rat prior to her attack was from house 842 on 14th April.

Connected cases—No. 11.

*Case 7.*

Umrudin, male, aet. 35, Mochi (shoemaker). Residence—902, Kasel. Had not left the village for some weeks before attack. Attack—20th April, fever, 104° F.; left axillary bubo on 22nd, died 24th April.



*Rats*—Dead plague-infected rats from residence on 14th and 18th April.  
Connected cases—Nos. 8 and 9.

*Case 8.*

Jano, female, aet. 15, Mochi (shoemaker). Residence—901, Kasel. Came from a village in the Lahore district on 16th April. Frequently went to house No. 902 which adjoins and is occupied by relatives of her husband. Attack—24th April, fever and right inguinal bubo; died 4th May.

*Rats*—No plague rats at residence—but *vide* Case 7.  
Connected cases—Nos. 7 and 9.

*Case 9.*

Sultanbibbi, female, aet. 5, Mochi. Residence—901, Kasel. Has not left the village for some months. Attack—25th April, fever and right inguinal bubo. Died 27th April.

*Rats*—*Vide* Cases 7 and 8.  
Connected cases—Nos. 7 and 8.

*Case 10.*

Ruri, female, aet. 35. Residence—897, Kasel. Had not left the village for many months before attack. Attack—25th April, fever and left inguinal bubo. Died 29th April.

*Rats*—No plague rats at residence; nearest infected rats from house No. 896 on 7th, and No. 894 on 11th April.  
Connected cases—nil.

*Case 11.*

Alla Ditta, male, aet. 30, Mahomedan Kamboh. Residence—857, Kasel. Went to Majitha on 18th April to see his mother (Case 6), who was reported to be ill with plague there. He returned to Kasel alone next day, but again went to Majitha on 21st April and returned to Kasel on 22nd bringing his mother (Case 6) back with him. Attack—27th April, fever. Seen on 28th, temperature 103° F.; no bubo. Developed a cough with rusty expectoration on night of 29th. Temperature on 30th, 104° F.; expectoration copious and rusty. Died on 1st May. Sputum showed swarms of *B. pestis*-like organisms and a guinea-pig inoculated cutaneously died of typical plague.

*Rats*—No plague rats at residence, but several from houses 842, 843 and 869 in vicinity between 14th and 22nd April.

Guinea-pig experiment—negative.  
Connected cases—No. 6.

*Case 12.*

Jhadoo, male, aet. 20, Bharai (beggar caste). Residence—246, Kasel (but 246, 247 and 248 are really one house). Had not left the village for a long time. When a plague rat was found in his house on 20th April, Jhadoo with his family moved into a neighbouring house, No. 263. Attack—28th April, fever and right femoral bubo; convalescent on 5th May; bubo subsided without suppuration.

*Rats*—Plague rats from residence (246) on 20th and 24th April.  
Connected cases—Case 43 in same family.

## Case 13.

Mamon, female, aet. 40, Mahomedan Kamboh. Residence—838, Kasel. Attack—28th April, fever and right inguinal bubo. Developed secondary plague pneumonia on 4th May. Premature delivery on 5th and died on 6th May. Sputum showed microscopically abundant bacilli like *B. pestis*.

*Rats*—None at residence but numerous plague rats in vicinity and a putrid rat from the next door house on 28th April.

Connected cases—nil.

## Case 14.

Rakki, female, aet. 13, Mahomedan Kamboh. Residence—868, Kasel. Had not left the village for some time. Attack—30th April, fever and left femoral bubo. Died on 2nd May.

*Rats*—A plague rat found in the house immediately adjoining on 17th April.

Connected cases—nil.

## Case 15.

Kisso, female, aet. 35, Hindu Nai. Residence—81 and 82, Kasel. Had not left the village for some weeks. Attack—2nd May, fever and left inguinal bubo. Died 5th May.

*Rats*—A live plague rat from her house on 17th April and a dead plague rat on 23rd April.

Connected cases—Nos. 16 and 18.

## Case 16.

Rattoo, female, aet. 40, Hindu Nai. Residence—81, 82, Kasel. Attack—3rd May, fever and right femoral bubo. Died 6th May.

*Rats*—*vide* Case 15.

Connected cases—Nos. 15 and 18.

## Case 17.

Dhani, female, aet. 10, Chuhra (sweeper). Residence—1243, Kasel. Had not left the village. Had gone daily to sweep out the courtyard of house No. 249, where a plague rat was found on 16th April. Attack—4th May, fever and left inguinal bubo. Seen on 6th May, temperature 99°, bubo very small and slightly tender. No abrasion of foot or leg could be found. Convalescent on 8th May. An attempt to obtain material from bubo on 6th May was unsuccessful; probably a case of *Pestis Minor*, but the diagnosis must be considered doubtful.

*Rats*—No plague rats at residence or anywhere in vicinity, but a plague rat at place of employment in house 249 (*vide supra*).

Guinea-pig experiments—negative.

Connected cases—nil.

## Case 18.

Tejo, female, aet. 13, Hindu Nai. Residence—83, Kasel. Had not left the village for many months. Frequently went to visit at adjoining house 81, 82, the occupants of which were her relatives. Attack—5th May, fever and right inguinal bubo; convalescent on 12th May, bubo subsiding without suppuration.

*Rats*—No plague rats at residence, but plague rats in adjoining house 81, 82, *vide* Cases 15 and 16.

Connected cases—15 and 16.

*Case 19.*

Dyali, female, aet. 45, Chuhra (sweeper). Residence—775, Kasel. Had not left Kasel for many months. Attack—7th May, fever and right inguinal bubo. Died 13th May.

*Rats*—No plague rats at residence, but plague rats from house No. 779, which is quite close, on 25th and 26th April, and a putrid rat from adjoining house, No. 776, on 6th May.

Connected cases—nil.

*Case 20.*

Bhano, female, aet. 20, Mazbhi Sikh. Residence—43, Kasel. Had not left the village prior to attack. Attack—7th May, fever, bubo in the neck on 8th; convalescent on 18th May, bubo subsiding without suppuration.

*Rats*—Two plague rats from residence on 27th April.

Connected cases—No. 33.

*Case 21.*

Basakhi, male, aet. 25, Mochi. Residence—902, Kasel. Arrived in Kasel on 30th April from the Lahore district on learning of the death of Case 7, who was a relative of his. Attack—8th May, fever and right femoral bubo. Was removed to his own village on 12th May where he recovered.

*Rats*—Plague rats at residence on 14th and 18th April.

Connected cases—No. 8.

*Case 22.*

Wiroo, male, aet. 40, Arora (shopkeeper caste). Residence—Lived in a neighbouring village, Mianpur, but had a shop at No. 78, Kasel, where he came daily. The shop was kept by three brothers who slept in it by turns. Attack—fever on 8th May. Seen on 9th when his temperature was 102·2°, on 10th 103°, and on 11th 98·4° F. Patient was removed to his house at Mianpur on 12th May. He was seen there on 18th when he was found to have a left femoral bubo. Died on 19th May.

*Rats*—No history of dead rats at his house in Mianpur, although rats were dying in the village at the time he fell ill. Plague rats from house No. 81, 82, Kasel, which immediately adjoins his shop No. 78, on 17th and 23rd April.

Connected cases—nil.

*Case 23.*

Maghloo, male, aet. 80, Chuhra (sweeper). Residence—756, Kasel. Had not left Kasel recently. Attack—8th May, fever and right inguinal bubo. Died 12th May.

*Rats*—Plague rats from an adjoining house, No. 757, on 4th and 8th May.

Connected cases—nil.

*Case 24.*

Nihalo, female, aet. 30, Jat Sikh. Residence—135, Kasel. Attack—9th May, right femoral and inguinal buboes and fever. Convalescent on 18th May, bubo subsiding.

*Rats*—A putrid rat (unfit for diagnosis) at residence on 28th April.

Connected cases—Nos. 38 and 39, *vide* note after Case No. 39.

*Case 25.*

Karimbibi, female, aet. 7, Kamiar. Residence—881, Kasel, up to 24th April. On that date the family moved into house No. 882, which is close by, in consequence of a plague rat having been found in the former house on 22nd April. Attack—9th May, fever. Seen on 10th May when her temperature was 101·6° F. No bubo found on examination. On 12th May, temperature was 103·4° F.; on 13th, 105° F., and patient delirious. She died on the afternoon of 13th May without developing any bubo.

*Rats*—Plague rats from residence (house No. 881) on 22nd and 27th April.

Connected cases—nil.

*Case 26.*

Santoo, male, aet. 45, Chuhra. Residence—757, Kasel. Attack—9th May, fever, 104·6° F. and right femoral bubo. Temperature on 10th, 101·6° F., with marked slurring of speech. Convalescent on 11th May; bubo subsided without suppuration.

*Rats*—Dead plague rats from residence on 4th and 8th May.

Connected cases—nil.

*Case 27.*

Bhagan, female, aet. 40, Chuhra. Residence—776, Kasel. Attack—9th May, fever. Seen 10th May, temperature 101·6° F., small left femoral and inguinal buboes. Patient quite unconscious. Died on 12th May.

*Rats*—A putrid rat from residence on 6th May, and plague rats from house No. 779, which is quite near, on 22nd and 26th April.

Connected cases—nil.

*Case 28.*

Rajo, female, aet. 40, Jat Sikh. Residence—8, Kasel. Had not left the village prior to attack. Attack—9th May, right inguinal bubo and fever. Seen on 10th May, temperature 105° F. Died on 13th May.

*Rats*—Dead plague rats from residence after attack (13th May). Plague rats from houses Nos. 923 and 938, which immediately adjoins No. 8, on 9th and 11th May.

Connected cases—nil.

*Case 29.*

Lachmi, female, aet. 22, Brahmin. Residence—391, Kasel. Had not left the village for 5 months before attack. Attack—9th May, pain in right axilla. On 10th right axillary bubo, temperature 101·6° F. Gave birth to a full term living child on 13th and died on 14th May.

*Rats*—Dead plague rats from residence on 29th April and 2nd May, and from an adjoining house, No. 392, on 5th, 6th and 7th May.

Connected cases—32 and 41.

*Case 30.*

Chuhru, male, aet. 2, Chuhra. Residence—759, Kasel. Had not left the village since birth. Attack—10th May, fever and left axillary bubo. Died on 11th May.

*Rats*—A putrid rat from residence on 4th May. Plague rats from adjoining houses, Nos. 757, 758, and 769, between 25th April and 8th May.

Connected cases—nil.

*Case 31.*

Khem-Kaur, female, aet. 35, Jat Sikh. Residence—486, Kasel. Had frequently visited at house No. 422 prior to her illness. Attack—10th May, with fever. First seen on 15th when her temperature was 102·4° F. No bubo found on examination. On 16th temperature was 102·8° F., on 17th 103·2° F., and patient was unconscious. On this date a small blister, about the size of a sixpence, was noticed below the right external malleolus. The relatives would not allow us to take material from the blister for bacteriological examination. Patient died on 17th May.

*Rats*—None at residence or in the vicinity till a month later. Several plague rats from house No. 422 (where patient used to visit) on 5th and 7th May.

Connected cases—nil.

*Case 32.*

Atma Ram, male, aet. 35, Brahmin, husband of Case 29. Residence—391, Kasel. Attack—11th May, fever, on 12th temperature 101·6° F., no bubo. Inguinal bubo appeared on 14th, temperature 101° F. Convalescent on 15th May; bubo subsided without suppuration.

*Rats*—*vide* Case 29.

Connected cases—Nos. 29 and 41.

*Case 33.*

Gango, female, aet. 20, Mazbhit, sister-in-law of Case 20. Residence—43, Kasel. Attack—11th May. Examined on 12th. Temperature 101·4° F.; right inguinal bubo, a small papule noticed below right anterior superior iliac spine which patient said had appeared on 10th May. This subsequently developed into a small carbuncle. Patient convalescent on 17th May.

*Rats*—*vide* Case 20.

Connected case—No. 20.

*Case 34.*

Gulabi, female, aet. 35, Jat Sikh. Residence—256, Kasel. Attack—11th May. Case examined 13th May, temperature 104·4° F. and right inguinal bubo. Died on 14th May.

*Rats*—A plague rat from residence on 15th May. Numerous plague rats from house No. 256, which almost adjoins No. 256, between 5th May and 12th May.

Connected cases—nil.

*Case 35.*

Durgo, female, aet. 8, Brahmin. Residence—422, Kasel. Had not left the village for several months before attack. Attack—12th May. Examined on 14th May, temperature 103·2° F., left axillary bubo. Died on 17th May.

*Rats*—Plague rats from residence on 5th and 7th May and a putrid rat on 8th May.

Connected cases—nil.

*Case 36.*

Alpal, male, aet. 40, Chuhra. Residence—767, Kasel. Attack—12th May. Examined the same day, temperature 102° F., right inguinal bubo. Died 16th May.

*Rats*—A putrid rat from residence on 14th May. Plague rats from house No. 769, which is quite near, on 1st May.

Connected cases—nil.



## Case 37.

Hazara Singh, male, aet. 7, Jat Sikh. Residence—225, Kasel. Attack—12th May, fever. Examined 13th May, temperature 106° F., no bubo found. On 14th patient delirious, temperature 102° F. Died at midnight without developing a bubo.

*Rats*—No plague rats at residence. Plague rat from an adjoining house, No. 218, on the 6th May and from No. 216 on 12th May.

Connected cases—nil.

## Case 38.

Jaro, female, aet. 7, Jat Sikh. Residence—133, Kasel. Attack—14th May, fever and bubo. Examined 14th May, temperature 103° F. Right femoral and right posterior cervical buboes. Convalescent on 17th May.

*Rats*—No plague rats at residence. A putrid rat from adjoining house, No. 135, on 28th April, and a dead plague rat in lane adjoining residence on 15th May.

Connected cases—Nos. 24 and 39.

## Case 39.

Khemi, female, aet. 30, Jat Sikh. Residence—134, Kasel, in house immediately adjoining No. 133 and in same courtyard. Attack—12th May, fever and right femoral bubo. Examined on 13th, temperature 102° F. Convalescent on 21st May; bubo subsided without suppuration.

*Rats*—*vide* Case 38.

Connected cases—Nos. 24 and 38.

The occupants of houses Nos. 133, 134 and 135 are three brothers and their families, and it appears that Cases 38 and 39 frequently went to house 135 while Case 24 was ill there.

## Case 40.

Jioni, female, aet. 25, Mahomedan Kamboh. Residence—818, Kasel. This woman left Kasel on 9th May accompanied by Bhagi (*vide* Case 46) to attend the mourning ceremonies of a relative who had died 7 days previously in a village, named Singhpur, in the Lahore District. This relative is said to have died from gout. Jioni and Bhagi returned together to Kasel on 10th May. Attack—12th May. Examined on 13th May, temperature 101° F., left inguinal bubo. Died on 14th May.

*Rats*—No plague rats from residence. Two putrid rats from a neighbouring house (No. 827) on 29th April and plague rats from houses Nos. 806 and 807 on 1st and 2nd May.

Connected cases—no evidence could be obtained as to whether this case was exposed to infection at Singhpur. The fact of both Jioni and Bhagi (Case 46) having gone to Singhpur together would suggest that they had been infected there. On the other hand Case 46 was not attacked till six days after her return from Singhpur, and again plague rats had been found in the vicinity of the houses of both cases in Kasel.

## Case 41.

Har Kour, female, aet. 50, Brahmin. Residence—391, Kasel. This patient came from a village, called Boparam, in the Amritsar District, about the 24th April to look after Lachmi (Case 29), who was then nearing her confinement. Attack—

13th May, fever and left femoral bubo. Seen on 14th, temperature 105° F., on 15th, 104·4° F. Patient was removed to her own house on 16th April and died on the way there.

*Rats*—*vide* Case 29.

Connected cases—Nos. 29 and 32.

*Case 42.*

Matharoo, male, aet. 7, Khatri (shopkeeper caste). Residence—158, Kasel. Previous to attack he went almost daily to his brother's shop, No. 255, Kasel. He was inoculated against plague on 9th May. Attack—bubo and fever on 13th May. Examined 14th May—temperature 102·8° F., right inguinal bubo. A left inguinal bubo appeared on 17th. Convalescent on 21st May, both buboes subsiding.

*Rats*—No plague rats at residence, but a dead plague rat from No. 159, a house adjoining his residence, on 10th May. Numerous plague rats from his brother's shop, No. 255, between 5th and 12th May.

Connected cases—nil.

*Case 43.*

Sammooo, male, aet. 8, Bharai (beggar tribe). Residence—246 and 247, Kasel; but was living temporarily in 263, Kasel when attacked. This boy left Kasel soon after his family moved into house No. 263 (*vide* Case 12), namely about the 25th April. He stayed with his uncle in a village, named Chak, in the Tarn Taran Tahsil, up to 9th May, when he returned to Kasel. On his return he stayed with his family in house No. 263, and it is said that he did not go into his own house (246, 247) after his return to Kasel. Attack—14th May. Examined 15th May, temperature 103·2° F., left cervical bubo; 16th May, temperature 101·2° F., convalescent on 21st May, bubo subsiding.

*Rats*—Plague rats at former residence, viz., house No. 246, 247 (*vide* Case 12). No plague rats from house No. 263, but a dead plague rat from No. 250, immediately adjoining, on 2nd May.

Connected cases—No. 12.

*Case 44.*

Ranga, male, aet. 35, Mahomedan Kamboh. Residence—823, Kasel. Attack—15th May. Examined 17th, temperature 100·2° F. and left axillary bubo. 18th, temperature 98·8° F. Convalescent on 20th May, bubo subsiding.

*Rats*—Plague rats from residence on 30th April and 3rd May.

Connected cases—nil.

*Case 45.*

Laldin, male, aet. 30, Mochi (shoemaker). Residence—908, Kasel, and works in an adjoining room, No. 909. Attack—15th May, pain in left femoral glands. Examined 16th May, temperature 100·8° F., left femoral bubo. 17th, temperature 104·8° F. Died on 18th May.

*Rats*—No plague rats from residence, but numerous plague rats from house 255 between 5th and 12th May. House No. 909 opens by a small door on to the street opposite No. 255.

Connected cases—nil.

## Case 46.

Bhagi, female, aet. 40, Mahomedan Kamboh. Residence—855, Kasel. For movements prior to attack, *vide* Case 40. Attack—16th May, bubo and fever. Examined 17th May, temperature 103° F., left inguinal bubo; 18th, temperature 103·6° F.; convalescent 24th May, bubo subsiding.

*Rats*—Plague rats from house No. 861 in the vicinity on 10th May.

Connected cases—nil.

## Case 47.

Basant-Kaur, female, aet. 25, Jat Sikh. Residence—85, 86, Kasel. Attack—16th May. Examined 17th May, temperature 101·4° F., left femoral bubo. Died 25th May.

*Rats*—A putrid rat from house No. 87, in the same courtyard and immediately opposite residence, on 9th May. Plague rat from house No. 94 in the immediate vicinity on 11th May.

Connected cases—nil.

## Case 48.

Gujjari, female, aet. 45, Chuhra. Residence—758, Kasel. Attack—16th May. Examined 17th, temperature 102·8° F., right axillary bubo; a small recent scar seen on front of right forearm said to be due to a burn. Convalescent on 21st May.

*Rats*—A plague rat from residence on 7th May and plague rats from the adjoining house (No. 757) on 4th and 8th May.

Connected cases—nil.

## Case 49.

Jiwan, female, aet. 30, Chuhra. Residence—748, Kasel. Attack—16th May. Examined 17th, temperature 102·6° F.; left inguinal bubo; 18th, temperature 104° F.; patient gave birth to a child (about a week before expected term). Died on 19th May.

*Rats*—None found at residence. A putrid rat from adjoining house, No. 750, on 16th May, and a plague rat from house No. 752 on 11th May and several recent plague rats from other houses in the vicinity.

Connected cases—nil.

## Case 50.

Jagga Singh, male, aet. 16, Jat Sikh. Residence—199, Kasel. Attack—17th May. Examined same day, temperature 102·6° F., right cervical bubo; 18th, temperature 99° F.; 19th, temperature 98·4° F., a second bubo in left femoral region appeared this day; 20th, temperature 104° F. Died on 23rd May.

*Rats*—A plague rat from residence on 24th May. Plague rats from house No. 181 in the immediate vicinity on 13th and 14th May.

Connected cases—nil.

## Case 51.

Biban, female, aet. 30, Mochi. Residence—936, Kasel. Attack—17th May. Examined 18th, temperature 100·2° F., left femoral bubo. Convalescent on 19th, bubo subsided.

*Rats*—No plague rats at residence, but a plague rat on 8th May from house No. 935 in the adjoining courtyard.

Connected cases—No. 64.

*Case 52.*

Gabo, female, aet. 7, Chuhra. Residence—749, Kasel. Attack—17th May. Examined 18th May, temperature 103° F., right inguinal bubo. Died 25th May.

*Rats*—No plague rats from residence, but a putrid rat from adjoining house, No. 750, on 16th, and a plague rat from house No. 752 on 11th May.

Connected cases—nil.

*Case 53.*

Jawali, female, aet. 30, Brahmin. Residence—398, Kasel. Attack—18th May. Examined on same date, temperature 102° F., spleen much enlarged and tender, no bubo admitted. On 19th, temperature 100° F., on 20th, 101° F., on 21st, 103·6° F., and delirious. Died on 21st May.

*Rats*—No plague rats from residence. Putrid rats from house No. 397, which immediately adjoins residence, on 9th May, and numerous plague rats from house No. 395 immediately opposite, between 9th and 20th May.

Connected cases—nil.

*Case 54.*

Natho, female, aet. 50, Kamboh. Residence—961, Kasel. This woman is a relative of the occupants of house No. 981. As far as could be ascertained she did not visit at this house, but one of the inmates of No. 981 (Khaardin) used to visit frequently at house 961 (*vide* Case 57). Attack—18th May, fever. Examined 20th, temperature 100·2° F., left femoral bubo; 21st, temperature 103·6° F. Convalescent on 30th May.

*Rats*—A plague rat from residence on 15th April; no plague rats from the immediate vicinity since 23rd April, but a plague rat from house No. 861 on 10th May, and one from house No. 953 on 5th May. As mentioned above, Khaardin, from house No. 981, was a constant visitor at her house, No. 961, at a time when plague rats were being found in the former house. Khaardin was himself attacked with plague on 20th May.

Connected case—No. 57.

*Case 55.*

Barkate, female, aet. 10, Kamboh. Residence—979, 980, Kasel. Inoculated against plague on 11th May. Attack—19th May. Examined on same date, temperature 102·2° F., left femoral bubo; 20th, temperature 102·2° F.; 21st, temperature 101° F.; 22nd, 98·4° F. Patient convalescent on 22nd May, bubo subsiding.

*Rats*—Plague rats from residence on 8th, 10th, and 12th May.

Connected cases—56 and 57, who are relatives living in the adjoining house, and both of whom came into contact daily with Case 55.

*Case 56.*

Ruckamdin, male, aet. 16, Kamboh. Residence—981, Kasel. Attack—19th May. Examined 21st, temperature 103° F., left femoral bubo; 22nd, temperature 104·4° F. Died 23rd May.

*Rats*—Plague rats from residence on 8th, 9th, and 11th May.

Connected cases—55 and 57.

*Case 57.*

Khairdin, male, aet. 40, Kamboh. Residence—981, Kasel. Attack—20th May. Examined 22nd, temperature 101·6° F., right femoral bubo; 23rd, temperature normal, convalescent. Bubo subsided.

*Rats*—*vide* Case 56.

Connected cases—Nos. 54, 55 and 56.

*Case 58.*

Bholi, female, aet. 30, Chuhra. Residence—754, Kasel. Attack—22nd May. Examined 23rd, temperature 100° F., left femoral bubo; 24th, temperature normal, convalescent. Bubo subsided.

*Rats*—A putrid rat from residence on 17th May and a plague rat from house No. 752 on 11th May.

Connected cases—nil.

*Case 59.*

Harkaur, female, aet. 20, Jat Sikh. Residence—102, Kasel. Attack—22nd May. Examined 23rd May, temperature 102° F., right femoral bubo; 24th, temperature 103° F. Died 28th May.

*Rats*—A plague rat from residence on 8th May and two putrid rats on 9th May.

Connected cases—nil.

*Case 60.*

Basant Kaur, female, aet. 25, Jat Sikh. Residence—244, Kasel. Attack—23rd May. Examined 24th May, temperature 101·8° F., left inguinal bubo; 25th, temperature 104° F. Convalescent on 1st June. Bubo suppurated.

*Rats*—A plague rat from residence on 12th May.

Connected cases—nil.

*Case 61.*

Hasain Mahommed, male, aet. 3, Jal. Residence—672, Kasel. Attack—22nd May. Examined 23rd May, temperature 101° F., parotid swellings on both sides resembling mumps. Swellings not hard and not very tender; 24th, temperature 98° F.; did not appear very ill, but died suddenly in the evening. Clinically the disease had all the appearance of mumps. No material was obtained for bacteriological examination; but, in view of the rapidly fatal issue, we consider the case highly suspicious, and have included it among the cases of plague.

*Rats*—No plague rats at residence or in the vicinity till some time after.

*Case 62.*

Tabo, female, aet. 30, Kamboh. Residence—917, Kasel. This woman lived with her husband in Hoshiarnagar, a village about three miles from Kasel. She came to visit her mother at No. 917, Kasel, during the first week in May and remained there up to 21st May, when she returned to her husband. On the day following her return to Hoshiarnagar she is said to have got fever, and a few days later developed a bubo in the right groin. She was brought back to a farm house about one mile from Kasel on 27th May. Examined on 28th May, temperature 101° F., right inguinal bubo.



*Rats*—No plague rats from residence in Kasel, but two plague rats from house No. 920, which is the next house but one, on 13th and 15th May. Plague existed in Hoshiarnagar from about the middle of April, and it was learned that a relative of Tabo's husband had died of plague there about the time she came to Kasel.

*Case 63.*

Kisso, female, aet. 18, Chuhra. Residence—744, Kasel. Attack—27th May. Examined 28th, temperature  $104.8^{\circ}$  F., left femoral bubo; 29th, temperature  $104.2^{\circ}$  F. Patient was removed to another village on this date, and it was subsequently learned that she recovered.

*Rats*—Plague rats from residence on 11th, 18th and 19th May.

Connected cases—nil.

*Case 64.*

Nathoo, male, aet. 10, Mochi. Residence—923, Kasel. This boy with his family vacated their house, No. 923, on 10th May in consequence of their finding a plague rat there on 9th May, and went to live in house 936, where Case 51 was attacked on 17th. The family returned to house 923 on 26th May. Attack—28th May. Examined 29th, temperature  $104.2^{\circ}$  F., right axillary bubo; 30th, temperature  $103.2^{\circ}$  F.; 31st, normal, bubo subsiding.

*Rats*—Plague rats from house 923 on 9th and 11th May. For rats in vicinity of house No. 923, *vide* Case 51.

Connected case—No. 51.

*Case 65.*

Jawali, female, aet. 40, Jat Sikh. Residence—230, Kasel, which is a "chaubara" (upper room) built on the roof of house No. 233. Previous to her attack she went daily to house No. 232, 233, where she had relatives. Attack—30th May. Examined 1st June, temperature  $102^{\circ}$  F., left inguinal bubo and a small phlyctenule on left buttock; 2nd June, temperature  $102.2^{\circ}$  F., phlyctenule had become a carbuncle; 3rd June, temperature  $102.2^{\circ}$  F., carbuncle large and very painful; 4th June, temperature  $102.6^{\circ}$  F., a crucial incision was made into carbuncle; 5th June, temperature  $102.6^{\circ}$  F.; 6th June, temperature  $98.4^{\circ}$  F., convalescent; bubo subsided without suppuration. Bacteriological examination of serum from the phlyctenule-smears and cultures—positive.

*Rats*—No plague rats at residence, but plague rats, on 7th and 23rd May, from house 232, 233, where case visited. Numerous plague rats from No. 395, which adjoins residence, between 9th and 20th May.

Connected cases—nil.

*Case 66.*

Jaina, female, aet. 16, Mahomedan Teli (oil extractor caste). Residence—175, Kasel. Attack—31st May. Examined 2nd June, temperature  $102^{\circ}$  F., left femoral bubo; 3rd June, temperature  $104^{\circ}$  F., cervical buboes on both sides, which appeared during the night. Died on 4th June.

*Rats*—No plague rats at residence, but from adjoining houses, Nos. 156 and 159, on 17th and 10th May respectively.

Connected case—No. 74 in same house, but not attacked till 26th June.

## Case 67.

Har Kour, female, aet. 80, Jat Sikh. Residence—no fixed abode for many months, though she owns house No. 207, which she went to after attack. This case used to go from house to house, and it is difficult to trace her movements prior to her illness. As far as could be ascertained, she lived in the courtyard of house No. 63, Kasel, till the 31st May. On that date she is said to have got fever, and was asked to go elsewhere. She went in the afternoon of 31st May to house No. 734 and remained there till 2nd June, when she was removed to her own house, No. 207. Attack—probably on 31st May. Examined on 3rd June, temperature 100·6° F., cough and copious expectoration of blood-tinged sputum. Died in the afternoon. Sputum examined microscopically and found to contain abundant organisms like *B. pestis*.

*Rats*—A putrid rat from house 63 on 8th May. Plague rats at house 734 on 30th May.

Connected cases—not ascertained.

## Case 68.

Ramzano, female, aet. 12, Mahomedan Fakir (beggar tribe). Residence—492, Kasel. Attack—8th June. Examined 9th June, temperature 104·2° F., left axillary bubo, delirious; 10th June, temperature 104·2° F. Died on 11th June.

*Rats*—A plague rat at residence on 8th June. A plague rat from house 493, immediately adjoining, on 4th June, and from No. 490 in the same courtyard on 31st May.

Connected case—No. 69.

## Case 69.

Budho, female, aet. 7, Mahomedan Fakir. Residence—1147, Kasel. This child was a relative of Ramzano (Case 68), and frequently visited at the latter's house (No. 492) both before and during her illness, and attended her funeral party on 11th June. Attack—11th June. Examined 12th, temperature 104·4° F., right inguinal bubo, unconscious; 13th, temperature 104·4° F.; 14th, temperature 100° F.; 15th, temperature 102·8° F. Died on this date.

*Rats*—None at residence or in its vicinity. Plague rats at house No. 492 (*vide* Case 68).

## Case 70.

Bahadur, male, aet. 7, Chuhra. Residence—745, Kasel. Attack—13th June. Examined 14th, temperature 102·8° F., right cervical bubo; 15th, temperature normal, convalescent, bubo subsided.

*Rats*—Several plague rats from residence, the last on 28th May.

Connected cases—nil.

## Case 71.

Gulab, male, aet. 40, Kamboh. Residence—270, Kasel. Attack—14th June. Case examined on 16th, temperature 103·2° F., left femoral bubo; 17th, temperature 101·2° F.; 18th, temperature normal, convalescent. Bubo subsided.

*Rats*—No plague rats at residence. A live plague rat from an adjoining house (272) on 16th June.

Connected cases—nil.

*Case 72.*

Gulab Fatma, female, aet. 3, Moh. Chhumba (washerman caste). Residence—662, Kasel. Attack—19th June. Examined 21st June, temperature 104·2° F., left femoral bubo; 22nd, temperature 103·2° F. Died on this date.

*Rats*—No plague rats at residence or in its vicinity previous to attack; plague rats from house 700 on 23rd and 26th June.

Connected cases—nil.

*Case 73.*

Fazal-bibi, female, aet. 16, Teli. Residence—667, Kasel. Attack—19th June. Case examined on 21st, temperature 104·2° F., right femoral bubo; 22nd, temperature 102° F. Convalescent on 28th June, bubo subsiding.

*Rats*—No plague rats at residence and no recent plague rats in the vicinity prior to attack, but plague rats from an adjoining house, No. 700, on 23rd and 26th July.

Connected cases—nil.

*Case 74.*

Tuli, male, aet. 7, Teli, brother of Case 66. Residence—175, Kasel. Is a relative of occupants of house No. 211, 212, Kasel, and frequently goes there. Attack—26th June, fever and right femoral bubo. Examined 28th June, temperature normal, and a small tender gland in right femoral region. Patient was convalescent on this date, and bubo subsided in a few days. No abrasion or wound could be found to account for the femoral swelling.

*Rats*—A live plague rat from house No. 138 in the vicinity of residence on 15th June. Plague rats on 31st May and 7th June at house 211, 212, where case used to visit.

Connected case—No. 66 in same house.

*Case 75.*

Assa Singh, male, aet. 30, Jat Sikh. Residence—620, Kasel. Attack—6th July, with fever, headache, and a bubo in right groin. First seen on 8th July, when his temperature was normal. A small, slightly tender right femoral bubo found on examination. No abrasion that would account for the bubo found. Patient went to work on this date, and bubo quickly subsided.

*Rats*—No plague rats from residence, but one from house No. 615 in the same compound on 15th June.

Connected cases—nil.

TABLE XXVIII.  
*Experiments with guinea-pigs running free in plague houses.*

Serial No.	Date	Address	No. of guinea-pigs put in	No. of fleas caught on guinea-pigs	No. of guinea-pigs which died of plague	Remarks
1	30/1/06	347 Dhand	2	48	—	Live plague rats caught on 27/1/06. One of the guinea-pigs died under chloroform, on this 40 of the fleas were found
2	1/2/06	"	1	30	—	Kept in back room of house No. 347, which had remained closed for more than a year
3	2/2/06	156 Dhand	1	12	—	Live plague rat on 30/1/06
4	2/2/06	342 Dhand	1	2	—	Live plague rat on 30/1/06
5	9/2/06	352 Dhand	1	16	—	Plague case on 6/2/06
6	9/2/06	349 Dhand	1	5	1	Plague case on 7/2/06
7	10/2/06	350 Dhand	1	16	1	Dead plague rat on 9/2/06
8	15/2/06	498 Dhand	1	—	—	Plague case on 13/2/06, but probably infected in house No. 350
9	15/2/06	340 Dhand	1	2	—	Plague case on 12/2/06
10	15/2/06	338 Dhand	1	—	—	Plague case on 13/2/06: guinea-pig put in upper room where case lived
11	21/2/06	"	1	—	—	Guinea-pig put in bhoosa godown in ground floor
12	21/2/06	181 Dhand	1	1	—	Plague case on 16/2/06
13	23/2/06	378 Dhand	1	7	1	Plague case on 22/2/06: guinea-pig put in back room
14	25/2/06	"	1	2	—	Two more plague cases on 24/2/06: guinea-pig put in living room
15	24/2/06	439 Dhand	2	4	—	Plague rat dead on 23/2/06
16	8/3/06	335 Dhand	2	2	—	Plague case on 5/3/06
17	16/3/06	746 Kasel	2	28	—	Imported plague case on 12/3/06
18	24/3/06	159 Dhand	2	26	—	Plague case on 22/3/06
19	27/3/06	357 Dhand	2	—	—	Suspicious case of plague on 25/3/06
20	29/3/06	402 Dhand	1	9	1	Dead plague rat on 28/3/06
21	31/3/06	39 Dhand	2	108	2	Plague case on 28/3/06
22	1/4/06	15 Dhand	1	25	1	Dead plague rats on 24/3/06 and 31/3/06: house vacated on latter date
23	3/4/06	122 Kasel	1	9	—	Dead plague rat on 2/4/06

24	4/4/06	114 Kasel	1	70	1	Dead plague rats on 3/4/06 and 4/4/06
25	7/4/06	68 Kasel	1	—	1	Plague case on 5/4/06
26	9/4/06	3 Dhand	1	—	—	Plague case on 7/4/06
27	10/4/06	225 Dhand	1	8	—	Plague cases on 4/4/06 and 7/4/06, but <i>vide</i> Table IV, No. 15
28	12/4/06	622 Dhand	1	7	—	Plague cases (2) on 10/4/06
29	17/4/06	589 Dhand	2	—	—	Plague case on 14/4/06
30	17/4/06	1010 Kasel	1	60	—	Plague case on 15/4/06
31	20/4/06	587 Dhand	1	—	—	Plague case on 14/4/06
32	21/4/06	583 Dhand	1	11	—	Live plague rat on 1/4/06: plague case on 19/4/06. Guinea-pig died under chloroform, but another guinea-pig on which its fleas were fed died of plague
33	23/4/06	1158 Kasel	1	9	—	Plague case on 18/4/06, but probably infected in another village
34	25/4/06	588 Dhand	1	1	—	Plague case on 20/4/06
35	25/4/06	220 Dhand	1	136	1	Plague case on 23/4/06
36	26/4/06	2 Dhand	1	1	—	Suspicious plague case on 21/4/06
37	1/5/06	857 Kasel	1	3	—	Plague case (pneumonic) on 27/4/06, probably infected in another village
38	4/5/06	386 Dhand	1	8	—	Plague case on 1/5/06
39	4/5/06	387 Dhand	1	30	—	Plague case on 2/5/06
40	7/5/06	1243 Kasel	1	—	—	Suspicious plague case on 4/5/06, probably infected elsewhere in the village
41	28/5/06	672 Kasel	1	—	—	Suspicious case on 22/5/06, clinically like mumps, but rapidly fatal
42	15/6/06	1147 Kasel	1	2	—	Plague case on 11/6/06, but probably infected in house No. 492
43	15/6/06	745 Kasel	1	5	—	Plague rat dead on 28/5/06, plague case on 13/6/06
44	17/6/06	270 Kasel	1	2	—	Plague case on 14/6/06
45	22/6/06	662 Kasel	1	3	—	Plague case on 19/6/06
46	25/6/06	667 Kasel	1	5	—	Plague case on 19/6/06
47	5/7/06	698 Kasel	1	—	—	Dead plague rat on 4/7/06: guinea-pig died on 12/7/06, not from plague
48	9/7/06	620 Kasel	1	—	—	Suspicious case of pestis minor on 7/7/06
49	10/7/06	669 Kasel	1	—	—	Dead plague rat on 9/7/06



V. EXPERIMENTS IN PLAGUE HOUSES IN DHAND  
AND KASEL.

In two papers already published (vol. VI. p. 467, vol. VII. p. 436) we have detailed several series of experiments which we carried out in plague houses in Bombay during the plague epidemics of 1906 and 1907. These observations went to prove, both directly and indirectly, that in a plague-infected house the infection is due to the presence therein of infected rat fleas, which are capable of transmitting the disease to animals.

Exactly similar series of experiments have been made in plague houses in Dhand and Kasel during the time plague was epidemic in these villages. We propose now to detail these observations, classifying them in the same way as has been done for Bombay. In the houses selected for experiment either a dead rat or rats had been found, which in many instances were proved to be plague infected at the laboratory, or a human case or cases had occurred or, in a few instances, both a dead rat had been found and a plague case had occurred.

The observations conveniently fall into three groups.

## GROUP I.

*Series I.**Experiments with guinea-pigs running free in plague houses.*

In this group guinea-pigs were allowed to run free for about eighteen hours in houses selected in the manner we have mentioned above. As a general rule only one guinea-pig was put into each house, but in a few instances two were used.

The details of the experiments of this series are contained in Table XXVIII.

This shows that out of 49 houses tested in this way nine, namely 18·4 %, were infective for the guinea-pigs. In all ten guinea-pigs contracted the disease. The distribution of the primary bubo in these ten animals was as follows: no bubo 2; neck alone 5; groin alone 2; neck and groin 1: so that in six out of eight cases with buboes the neck glands were affected.

Table XXIX shows the average number of fleas taken in each of four groups of houses:—A, houses which were infective for guinea-

pigs: B, houses which were not infective for guinea-pigs: C, houses which were proved to be plague infected, because a rat proved to be plague infected had been found in the house, or because the guinea-pig developed the disease, or because both a plague-infected rat had been found and the guinea-pig developed plague: D, houses not proved to be plague infected. It is seen that in the houses of groups A and C, especially in A, the number of fleas taken was very much greater than in the houses of groups B and D.

TABLE XXIX.

*Showing the results of the flea census in houses, classified according to their being proved plague infected or not.*

	Nature of houses	No. of houses	No. of fleas	Average No. of fleas per house
1.	Total houses	49	713	14.5
2.	A. Houses which were infective for guinea-pigs	9	376	42
3.	B. Houses which were not infective for guinea-pigs	40	337	8.4
4.	C. Houses which were proved plague infected	18	467	26
5.	D. Houses not proved to be plague infected	31	246	8

*Summary.*

In 18 % of instances guinea-pigs allowed to run free in plague houses developed the disease and died.

Three times as many fleas were caught in plague-infected as in not plague-infected houses, and five times as many in houses which proved infective to guinea-pigs as in those not infective.

GROUP II.

*Series II.*

*Experiments with fleas caught on plague-infected rats found in houses.*

In this series of experiments, only six in number, fleas, taken on rats which were proved by post-mortem and bacteriological examination to be plague infected, were transferred to healthy guinea-pigs in flea-proof cages in the laboratory. The rats were either found dead or trapped in a dying condition in houses in the villages. The details of these experiments are given in Table XXX. From this table it is seen that three out of six of the guinea-pigs to which the fleas were transferred died of plague.

TABLE XXX.  
*Experiments with fleas caught on plague-infected rats found in houses.*

Serial No.	Date	Address	Number of fleas	Species and number of animals on which fleas were fed	Number which died of plague	Remarks
1	26/2/06	163 Dhand	83	guinea-pig 1	1	83 fleas removed from two rats caught in one trap. Both rats died soon after arrival at the laboratory, only one proved to be plague infected
2	5/3/06	24 Dhand	4	guinea-pig 1	1	Fleas obtained from one dead plague rat found in a bhoosa store
3	6/3/06	178 Dhand	30	guinea-pig 1	1	Fleas obtained from one dead plague rat found in a godown of this house
4	31/3/06	137 Dhand	3	guinea-pig 1	—	Fleas obtained from one dead plague rat found in a bhoosa store
5	2/4/06	122 Kasel	176	guinea-pig 1	—	Fleas obtained from one dead plague rat found in this shop
6	9/4/06	119 Kasel	48	guinea-pig 1	—	Fleas obtained from one dead plague rat found in this house

*Series III.*

*Experiments with fleas caught on guinea-pigs and rats which had been left for some hours in plague houses.*

In this series of experiments fleas were caught either on guinea-pigs or on rats, which had been placed in plague houses for about eighteen hours. The fleas were brought to the laboratory in test-tubes. They were then immediately put on a fresh guinea-pig or rat in a flea-proof cage. The details of these observations are set forth in Table XXXI, which shows that in seven out of 25 experiments the animals, always guinea-pigs, to which the fleas were transferred, died of plague. The distribution of the primary bubo in these seven guinea-pigs was as follows: 1 no bubo; 4 in the neck alone; and 2 in the groin alone.

*Summary.*

In 10 out of 31 experiments fleas caught on rats and guinea-pigs in plague houses conveyed plague to fresh animals in the laboratory.

GROUP III.

*Series IV.*

*Experiments with animals in cages, unprotected and protected with fine wire gauze, placed in plague houses.*

In this series a curtain of fine metallic gauze, such as is used for filtering petrol, was used to prevent the access of fleas. In the first report we have already described and figured the cages which were employed for this purpose in Bombay. Cages of exactly the same pattern were used in the present series of experiments.

The details of the experiments are set forth in Table XXXII. From this table it is seen that twenty-one experiments were made, guinea-pigs being the only animals used.

Only on one occasion were any fleas got on the protected animals, and then only a single one. On the unprotected animals on seventeen occasions fleas were taken, as many as seventy-five being caught on one occasion. None of the protected animals contracted plague, while four (19 %) of the unprotected guinea-pigs died of the disease. Every one of these guinea-pigs had a bubo in the neck, and in the case of two of them there was also a bubo in the groin.

TABLE XXXI.

*Experiments with fleas caught on guinea-pigs and rats which had been left for a night in plague houses.*

Serial No	Date	Address	Number of fleas	Species and number of animals on which fleas were fed	Number which died of plague	Remarks
1	30/1/06	347 Dhand	70	Guinea-pig 1	—	Vide Nos. 1 and 2, Table XXVIII
2	9/2/06	352 Dhand	16	Guinea-pig 1	—	Vide No. 5, Table XXVIII
3	9/2/06	349 Dhand	5	Guinea-pig 1	—	Vide No. 6, Table XXVIII
4	10/2/06	350 Dhand	16	Guinea-pig 1	1	Vide No. 7, Table XXVIII
5	23/2/06	"	26	Rat 1	—	These fleas were got from a rat which was kept in a trap in back room of house No. 350 in which a plague rat was found on 9/2/06
6	23/2/06	349 Dhand	178	Rat 1	—	These fleas were got from 2 rats kept in a trap in the house
7	25/2/06	439 Dhand	2	Guinea-pig 1	—	Vide No. 15, Table XXVIII
8	16/3/06	746 Kasel	28	Guinea-pig 1	—	Vide No. 17, Table XXVIII
9	24/3/06	159 Dhand	26	Guinea-pig 1	—	Vide No. 18, Table XXVIII
10	29/3/06	402 Dhand	9	Guinea-pig 1	1	Vide No. 20, Table XXVIII
11	31/3/06	39 Dhand	108	Guinea-pig 1	1	Vide No. 21, Table XXVIII
12	1/4/06	15 Dhand	25	Guinea-pig 1	1	Vide No. 22, Table XXVIII
13	3/4/06	122 Kasel	9	Guinea-pig 1	—	Vide No. 23, Table XXVIII
14	4/4/06	114 Kasel	70	Rat 1	1	Vide No. 24, Table XXVIII
15	10/4/06	225 Dhand	14	Guinea-pig 1	1	Vide No. 27, Table XXVIII
16	12/4/06	622 Dhand	5	Guinea-pig 1	—	The fleas were got from 1 rat and 1 guinea-pig kept for a night in the house. Neither of these animals died of plague.
17	17/4/06	1010 Kasel	60	Guinea-pig 1	—	Vide No. 28, Table XXVIII
18	21/4/06	583 Dhand	11	Guinea-pig 1	1	Vide No. 30, Table XXVIII
19	23/4/06	1158 Kasel	9	Rat 1	—	Vide No. 32, Table XXVIII
20	25/4/06	220 Dhand	100	Rat 1	—	The fleas were caught on a guinea-pig which died under chloroform during removal of fleas
21	4/5/06	387 Dhand	30	Guinea-pig 1	—	Vide No. 33, Table XXVIII
22	16/6/06	1147 Kasel	2	Guinea-pig 1	—	Vide No. 35, Table XXVIII
23	16/6/06	745 Kasel	4	Guinea-pig 1	—	Vide No. 39, Table XXVIII
24	17/6/06	270 Kasel	2	Guinea-pig 1	—	Vide No. 42, Table XXVIII
25	25/6/06	667 Kasel	5	Guinea-pig 1	—	Vide No. 43, Table XXVIII
						Vide No. 44, Table XXVIII
						Vide No. 46, Table XXVIII



TABLE XXXII.

*Experiments with animals in protected and unprotected cages placed in plague houses.*

Serial No.	Date	Address	Species of animals used	Number of fleas found on animals		Fate of animals		Remarks
				Protected	Unprotected	Protected Survived	Unprotected Survived	
1	20/4/06	248 Kasel	Guinea-pig	—	6	—	—	Dead plague rat found on 20/4/06
2	21/4/06	784 "	"	—	1	"	"	Dead plague rats on 19/4 & 20/4/06
3	21/4/06	782 "	"	—	75	"	"	Dead plague rat on 21/4/06
4	22/4/06	779 "	"	1	12	"	"	Dead plague rat on 22/4/06
5	30/4/06	769 "	"	—	2	"	"	Dead plague rats on 25/4/06, 30/4/06 & 1/5/06
6	3/5/06	807 "	"	—	6	"	"	Dead plague rats on 2/5/06 & 3/5/06
7	8/5/06	422 "	"	—	32	"	"	Three plague rats on 5/5/06 & two on 7/5/06
8	9/5/06	923 "	"	—	6	"	"	Dead plague rats on 9/5/06 & 11/5/06
9	9/5/06	980 "	"	—	30	"	"	Plague rats on 8/5/06, 10/5/06
10	14/5/06	256 "	"	—	4	"	"	Plague case on 11/5/06
11	"	920 "	"	—	—	"	"	Dead plague rat on 15/5/06
12	"	746 "	"	—	—	"	"	Dead plague rats on 13/5/06 & 15/5/06
13	16/5/06	376 "	"	—	4	"	"	Dead plague rat on 14/5/06
14	"	371 "	"	—	2	"	"	One dead plague rat on 16/5/06
15	19/5/06	743 "	"	—	4	"	"	Two dead plague rats on 17/5/06
16	"	745 "	"	—	—	"	"	One dead plague rat on 18/5/06
17	25/5/06	199 "	"	—	—	"	"	Dead plague rat on 15/5/06
18	9/6/06	212 "	"	—	1	"	"	Plague rats on 11/5, 18/5, 19/5 and 20/5/06
19	15/6/06	615 "	"	—	1	"	"	One dead plague rat on 16/5
20	"	1036 "	"	—	4	"	"	Two dead plague rats on 17/5
21	26/6/06	700 "	"	—	3	"	"	Dead plague rat on 24/5

*Series V.*

*Experiments with animals in cages, one surrounded with "tanglefoot" the other not so protected, placed in plague houses.*

Cages of exactly the same pattern as were used in the Bombay experiments and which have been already described and figured were employed for the present series. One of the animals instead of being protected from fleas by means of a metallic gauze curtain, was surrounded by an area spread with a sticky resinous preparation, called "tanglefoot," which in the case of the other animal was replaced by a layer of sand. By this contrivance it was expected that fleas, which were attempting to get at the animals, in the case of the "tanglefoot" cage would be caught on this material, but that in the case of the sand cage, with no such barrier to cross, would be able to get at the animal itself. At the same time both animals were protected from soil and contact infection and were equally exposed to aerial infection. In Table XXXIII are set forth the results of these observations.

Only in the first few experiments was any attempt made to identify the species of fleas caught on the tanglefoot and to dissect these, in order to ascertain whether plague bacilli were present in the stomach contents or not. At this time the epizootic and epidemic in Kasel were at their height and the large amount of work which was entailed in their study left little or no time for experimental work. It will be seen, however, that of 156 fleas, the species of which were noted, 138 were rat fleas and only 18 cat fleas. Further, it will be seen from the table that out of 55 rat fleas dissected the stomach contents of three were found to contain plague-like bacilli. 217 fleas which were neither identified nor dissected were also caught on the tangle-foot.

As regards the number of fleas taken on the animals themselves, only on three occasions out of 30 experiments were any fleas found on the animals which were surrounded with tanglefoot and on each of these animals only one flea was taken. As regards the sand cages, a total of 54 fleas were caught off 17 animals, while no fleas were found on the remaining 13 guinea-pigs.

None of the animals which were surrounded by tanglefoot developed plague; two of those left unprotected contracted the disease.

*Summary.*

In houses in which plague-infected rats had been found animals protected and unprotected from fleas were placed. In 51 experiments

Serial No.	Date	Address	Species of animal used	Species and number of fleas caught on tangle-foot	Species and number of fleas caught on tangle-foot with <i>X. pests</i>	Number of fleas caught on animals		Fate of animals		Remarks
						Tangle-foot	Non-Tangle-foot	Tangle-foot	Non-Tangle-foot	
1	20/4/06	960 Kasel	Guinea-pig	2 rat, 7 cat	5 dissected, none infected	—	—	survived	died under chloroform	Dead plague rat on 19/4/06
2	"	111	"	5 rat	5 rat - 2	—	2	"	survived	Dead plague rats on 18/4/06 & 19/4/06
3	23/4/06	969	"	66 rat	40 rat - 1	1	4	"	"	Dead plague rat on 23/4/06
4	30/4/06	823	"	10 rat, 4 cat	5 rat - 0	—	2	died under chloroform	"	Dead plague rat on 30/4/06
5	3/5/06	393	"	6 rat, 3 cat	none dissected	1	—	chloroform	"	Dead plague rat on 2/5/06
6	"	390	"	19 rat, 3 cat	"	1	10	"	"	Plague rats on 27/4, 29/4, 1/5 (2) & 2/5/06
7	5/5/06	422	"	30 rat, 1 cat	"	2	—	"	"	Vide No. 7, Table XXXII
8	8/5/06	51	"	—	—	—	—	"	chloroform on 24/5, plague survived	6 plague rats between 4/5 & 7/5/06
9	"	421	"	3 (species not noted)	none dissected	—	—	"	survived	Dead plague rat on 8/5/06
10	10/5/06	790	"	7 (species not noted)	"	—	6	"	died on 13/5/06, not plague	Dead plague rat on 7/5/06
11	14/5/06	181	"	19 (species not noted)	"	—	—	"	survived	Live plague rat on 9/5/06
12	"	395	"	114, mostly rat	"	—	12	"	died of plague 18/5	1 dead plague rat on 13/5/06
13	16/5/06	375	"	2 (species not noted)	"	—	—	"	survived	2 dead plague rats on 14/5/06
14	"	373	"	6 (species not noted)	"	—	2	"	"	Dead plague rats on 9/5 & 13/5/06
15	19/5/06	376	"	1	"	—	—	"	"	4 dead plague rats on 14/5/06
16	21/5/06	371	"	19	"	—	—	"	"	3 dead plague rats on 15/5/06
17	"	712	"	9	"	—	1	"	"	4 dead plague rats between 16/5 & 18/5
18	23/5/06	232	"	13	"	—	1	"	"	Dead plague rats on 15/5 & 20/5
19	22/5/06	224	"	1	"	—	1	"	"	Plague rats on 20/5, 21/5 & 22/5
20	25/5/06	375	"	8	"	—	—	"	"	Plague rat on 23/5/06
21	25/5/06	373	"	7	"	—	3	"	"	Plague rat on 22/5/06
22	27/5/06	355	"	2	"	—	—	"	"	Vide No. 13 supra
23	28/5/06	745	"	—	"	—	—	"	"	Putrid rat on 24/5/06
24	31/5/06	734	"	1 (species not noted)	"	—	1	"	"	Vide No. 14 supra
25	31/5/06	728	"	3	"	—	—	"	"	Dead plague rat on 20/5/06
26	2/6/06	223	"	2	"	—	1	"	"	Dead plague rat on 27/5/06
27	9/6/06	693	"	—	"	—	—	"	"	1 dead plague rat on 16/5/06
28	9/6/06	321	"	—	"	—	—	"	"	2 dead plague rats on 17/5/06
29	12/6/06	485	"	—	"	—	—	"	"	1 dead plague rat on 28/5/06
30	12/6/06	355	"	—	"	—	—	"	"	Dead plague rat on 30/5/06
										2 dead plague rats on 30/5/06
										Dead plague rat on 1/6/06
										Dead plague rat on 7/6/06
										Putrid rat on 8/6/06
										Dead plague rat on 10/6/06
										Vide No. 22 supra
										Dead plague rat on 11/6/06

fleas were found on the protected animals four times and on the unprotected 34 times; six of the unprotected animals died of plague but none of the protected guinea-pigs.

It is unnecessary to enter into the conclusions which may be drawn from these observations. We have already set them forth in detail in the two papers which have reference to the similar series of observations made in Bombay. It is, however, important to note that exactly the same results were obtained in these Punjab villages as in Bombay.

## VI. THE QUESTION WHETHER PLAGUE TENDS TO RECUR IN HOUSES IN SUCCESSIVE EPIDEMICS.

An attempt was made to determine whether houses, which were infected in one epidemic were especially liable to be again infected in any subsequent epidemic.

We had first to find out particulars of the incidence of plague (*i.e.* plague cases) in houses during previous epidemics.

A record of deaths from plague in previous epidemics existed and it was only necessary to determine accurately the houses in which these deaths had occurred. The information with regard to non-fatal cases, of which no records existed, was obtained by inquiry at each house, whether any of the family had recovered from plague and if so during which epidemic. Only cases in which a definite history of fever and bubo was given were taken as having had plague.

From the information derived from these two sources lists of the houses in which plague cases had occurred were prepared for each epidemic. The total number of occupied houses and the number of houses infected in each epidemic for both Dhand and Kasel is shown in Table XXXIV.

TABLE XXXIV.

*Showing the total number of occupied houses and the number of houses infected in each epidemic.*

Village	Total No. of occupied houses	No. of houses infected in first epidemic	No. of houses infected in second epidemic	No. of houses infected in third epidemic	No. of houses infected in fourth epidemic
Dhand	418	101	198	40	26
Kasel	806	308	252	230	67

Table XXXV shows the actual numbers of houses which were infected in one epidemic only, in any two, in any three and in all four

epidemics; while Table XXXVI gives the figures, which have been calculated from the data in Table XXXIV, showing the probable number of houses which would have been infected in one, two, three, and four epidemics, on the assumption that the houses were equally liable to infection throughout all four epidemics.

TABLE XXXV.

*Showing the actual number of houses in Kasel and in Dhand which were infected (i.e. which furnished plague cases) in one, two, three and four epidemics.*

Village	No. of houses infected			
	In one epidemic only	In any two epidemics	In any three epidemics	In all four epidemics
Dhand	208	65	9	0
Kasel	383	169	43	2

TABLE XXXVI.

*Showing the calculated probable number of houses which would have been infected in one, two, three and four epidemics, if all houses were equally liable to infection throughout all four epidemics.*

Village	No. of houses infected			
	In one epidemic only	In any two epidemics	In any three epidemics	In all four epidemics
Dhand	201	73	8	0·3
Kasel	393	162	40	2

The close correspondence between the actual and calculated figures suggests that the assumption on which the latter figures were worked out is a legitimate one, or, in other words, that plague showed no tendency to recur in houses during successive epidemics.



## CORRIGENDA.

Volume VII. p. 470, lines 6 and 34. *For* "in the Punjab" *read* "in the Punjab villages of Dhand and Kasel." In some parts of the Punjab acute human plague occurs in the off-plague season.

Case *Q*, in the same house as case *T*, in the northern part of Dhand, is marked *R* in Map 1: it appears as "4" in Map 12. The real case *R* is in the same house as *W* on Map 1.

## INDEX OF AUTHORS.

	PAGE
ADVISORY COMMITTEE. Reports on Plague Investigations in India [issued by the Advisory Committee] continued from vol. VI, p. 536). [Second Plague Number.]	
XI. The diagnosis of natural rat plague. (Plate VII.) . . . .	324
XII. The pathological histology of the spleen and liver in spontaneous rat-plague, with observations on the experimental infection. By J. C. G. Ledingham, M.B., B.Sc., M.A. (Plates VIII and IX.) . .	359
XIII. Transmission of plague by feeding rats with infected material .	373
XIV. On the significance of the locality of the primary bubo in animals infected with plague in nature . . . . .	382
XV. Further observations on the transmission of plague by fleas, with special reference to the fate of the plague bacillus in the body of the rat flea ( <i>P. cheopis</i> ) . . . . .	395
XVI. Experimental production of plague epidemics among animals. (One Chart.) ( <i>Second Communication.</i> ) . . . . .	421
XVII. Experiments in plague houses in Bombay. ( <i>Second Communication.</i> ) . . . . .	436
XVIII. On the external anatomy of the Indian rat flea ( <i>P. cheopis</i> ), and its differentiation from some other common fleas. (Plates X to XII.) . . . . .	446
XIX. On the natural occurrence of chronic plague in rats . . . .	457
XX. A note on man as a host of the Indian rat flea ( <i>P. cheopis</i> ) .	472
[Third Plague No. (Plates XIX to XLI with seventy-six maps and charts)]	
XXI. Digest of recent observations on the epidemiology of plague .	694
XXII. The epidemiological observations made by the Commission in Bombay City . . . . .	724
XXIII. Observations made in four villages in the neighbourhood of Bombay . . . . .	799
XXIV. General considerations regarding the spread of infection, infectivity of houses, etc. in Bombay City and Island . . . . .	874
XXV. Observations in the Punjab villages of Dhand and Kasel . .	895
ARKWRIGHT, J. A. On the Occurrence of the <i>Micrococcus catarrhalis</i> in Normal and Catarrhal Noses and its Differentiation from other Gram-negative Cocci . . . . .	145

	PAGE
ARKWRIGHT, J. A. On Variations of the Meningococcus and its Differentiation from other Cocci occurring in the Cerebro-Spinal Fluid . . . .	193
ARMIT, H. W. The Toxicology of Nickel Carbonyl. (Four Figures.) . . .	525
BARTON, <i>see</i> HEWLETT.	
BASSETT-SMITH, P. W. The Treatment of Mediterranean Fever by means of Vaccines, with Illustrative Cases. (Seventeen Charts.) . . . .	115
CASTELLANI, A. Notes on Cases of Fever frequently confounded with Typhoid and Malaria in the Tropics. (Three Charts.) . . . .	1
CASTELLANI, A. Experimental Investigations on <i>Framboesia tropica</i> (Yaws). (Plates XV and XVI and One Figure.) . . . .	558
CRAW, J. A. On the Danysz Effect with reference to the Toxin-Antitoxin Reaction. (One Figure.) . . . .	501
CRAW, J. A. On Variation in Weight of Normal Guinea-pigs in relation to the Estimation of Free Diphtheria Toxin . . . .	589
CRAW, J. A. and DEAN, G. On the Estimation of free Diphtheria Toxin: with reference to the relations existing between lethal doses, lethal times and loss in weight of the guinea-pig. (Two Figures.) . . . .	512
CUMPTON, H. A Contribution to the Bacteriology of Post-Scarlatinal Diphtheria . . . .	593
CUMPTON, H. The Relative Frequency of Various Types of Streptococci in Scarlatina . . . .	599
CURRIE, J. R. On the Supersensitisation of Persons suffering from Diphtheria by Repeated Injections of Horse Serum. (Three Diagrams.) . . .	35
CURRIE, J. R. Examples of the Immediate and of the Accelerated Reaction following Two Injections of Anti-diphtherial Serum . . . .	61
DEAN, <i>see</i> CRAW.	
DUDGEON, L. S., and DUNKLEY, E. V. The <i>Micrococcus neoformans</i> . .	13
DUNKLEY, <i>see</i> DUDGEON.	
FRÖLICH, <i>see</i> HOLST.	
GOODALL, E. W. On the Supersensitisation of Persons by Horse-serum . .	607
GRAHAM-SMITH, G. S. A Cystic Disease of the Heart, Gizzard and Muscles of Young Grass Parakeets ( <i>Psittacus undulatus</i> ) due to a Protozoan Parasite. (Plates XIII and XIV.) . . . .	552
GRAHAM-SMITH, <i>see</i> NUTTALL.	
GREEN, A. B. A Note on the Influence of the Chemical Rays of Daylight on Vaccinia in Animals . . . .	155
GREENWOOD, M., JUNR. and THOMPSON, T. On Meteorological Factors in the Aetiology of Acute Rheumatism. (Four Diagrams.) . . . .	171
HEWLETT, R. T. In MEMORIAM. ALLAN MACFADYEN. (Plate VI.) . . .	319
HEWLETT, R. T. and BARTON, G. S. The Results of a Chemical, Microscopical and Bacteriological Examination of Samples of London Milks .	22
HOLST, A. Experimental Studies relating to "Ship-beri-beri" and Scurvy. I. Introduction . . . .	619
HOLST, A. and FRÖLICH, T. Experimental Studies relating to "Ship-beri-beri" and Scurvy. II. On the Etiology of Scurvy. (Plates XVII and XVIII.) . . . .	634

	PAGE
IMMS, A. D. On the Larval and Pupal Stages of <i>Anopheles maculipennis</i> , Meigen. (Plates IV and V and One Figure.) . . . . .	291
LEDINGHAM, J. C. G. On the Relation of the Antitoxin to the Globulin-Content of the Blood Serum during Diphtheria Immunisation. (Four Charts.) . . . . .	65
LEDINGHAM, J. C. G. Notes on the Leucocyte-Reaction during the Immunisation of the Horse and Goat with Diphtheria Toxin . . . . .	92
MALDEN, W. Some Observations on the Condition of the Blood in Men engaged in Anilin Dyeing and the Manufacture of Nitrobenzine and its Compounds. (Two Charts.) . . . . .	672
MARSHALL, W. E. Note on the Occurrence of Diphtheria Bacilli in Milk . . . . .	32
MARSHALL, W. E. The Para-dimethyl-amido-benzaldehyde Test for Indole. (Three Charts.) . . . . .	581
MCCRAE, J. and STOCK, P. G. Some Experiments with Fluorescein as an Agent for the Detection of Pollution of Wells. (One Map.) . . . .	182
NOON, L. On the Occurrence of Toxic Compounds of Tetanus Toxin and Antitoxin, Tetanus Toxin and Brain Emulsions. (One Figure.) . . .	101
NUTTALL, G. H. F. and GRAHAM-SMITH, G. S. Canine Piroplasmosis. VI. Studies on the Morphology and Life-History of the Parasite. (Plates I, (II, III, and Fourteen Diagrams.) . . . . .	232
PAYNE, <i>see</i> REVIS.	
REVIS, C. and PAYNE, G. A. The Acid Coagulation of Milk. (Curves A, B, C.) . . . . .	216
SAVAGE, W. G. The Bacteriological Examination of Surface Wells . . . .	477
SMITH, J. H. On the Absorption of Antibodies from the Subcutaneous Tissues and Peritoneal Cavity. (Five Figures.) . . . . .	205
STOCK, <i>see</i> MCCRAE.	
THOMPSON, <i>see</i> GREENWOOD.	
TODD, C. Some Experiments on the Filtration of Cattle-Plague Blood. (Seven Charts.) . . . . .	570
WENYON, C. M. Action of the Colours of Benzidine on Mice infected with <i>Trypanosoma dimorphon</i> . . . . .	273

## INDEX OF SUBJECTS.

	PAGE
Air, <i>see</i> Carbon-monoxide, Nickel carbonyl	
Anilin dyeing, effect on blood condition ... ..	672
<i>Anopheles maculipennis</i> , the larva and pupa ... ..	291
Antibodies, absorption of in the body ... ..	205
Antitoxin for Diphtheria ... ..	35, 61, 92
,,     ,, tetanus ... ..	101
,,     relation to globulin in serum ... ..	65
Antitoxin-toxin reaction ... ..	501
<i>Bacillus ceylanensis</i> ... ..	8
,, <i>coli</i> , in surface wells and soil ... ..	480, 494
,,     ,, and allied forms compared ... ..	489
,, <i>diphtheriae</i> in milk ... ..	32
,,     ,, toxin estimation ... ..	512, 589
,,     ,, <i>see</i> Diphtheria	
,, <i>pestis</i> , <i>see</i> Plague bacillus	
Benzidine colour-therapy, <i>see</i> <i>Trypanosoma</i>	
Beri-beri, <i>see</i> Ship	
Blood, <i>see</i> Anilin, Antibodies, Antitoxin, Cattle-plague, Carbon monoxide, Nickel carbonyl, Nitro-benzine	
Bordet-Gengou reaction in Yaws ... ..	565
Carbon monoxide, toxicology of ... ..	528
Cattle-plague, filtration of blood in ... ..	570
Cerebro-spinal Meningitis, <i>see</i> Meningococcus	
Danysz effect, toxin-antitoxin reaction ... ..	501
Diphtheria antitoxin ... ..	35, 61, 65, 92
,,     ,, relation to serum-globulin ... ..	65
,,     post-scarlatinal ... ..	593
,, <i>see</i> <i>Bacillus</i>	
Dog, <i>see</i> <i>Piroplasma</i>	
Fevers in Ceylon ... ..	1
Flea, <i>see under</i> Plague	
Flies in relation to Yaws transmission ... ..	566
Fluorescein for detection of well pollution ... ..	182
Fowls, <i>see</i> Ship-beri-beri	
<i>Framboesia tropica</i> (Yaws) ... ..	558



	PAGE
Guinea-pigs, variation in weight ... ..	589
„ see Plague	
Immunity in Yaws ... ..	565
„ in Malta fever ... ..	115
„ see Antibodies, Antitoxin, Bordet, Light, Leucocytosis, Super-sensitation	
Indole, test for ... ..	581
Insects, see <i>Anopheles</i> , Flea, Flies	
Leucocytosis during immunisation with Diphtheria toxin ... ..	92
Light, effect on vaccinia in animals ... ..	155
Macfadyen, Dr Allan (In Memoriam, with Portrait) ... ..	319
Malarial mosquito, see <i>Anopheles</i>	
Malta fever, vaccine treatment ... ..	115
Mediterranean fever, see Malta	
Meningococcus, differentiation from other cocci ... ..	193
Meteorological factors, relation to acute rheumatism ... ..	171
<i>Micrococcus catarrhalis</i> in the nose, differentiation ... ..	145
„ <i>intracellularis</i> , see Meningococcus	
„ <i>melitensis</i> , see Malta	
„ <i>neoformans</i> ... ..	13
Milk, acid coagulation of ... ..	216
„ bacteriology of ... ..	22
„ diphtheria bacilli in ... ..	32
Monkey, see Yaws, Syphilis	
Mosquito, see <i>Anopheles</i>	
Mouse, see <i>Trypanosoma</i>	
Nickel carbonyl, solubility of, in serum and blood ... ..	538
„ „ toxicology of ... ..	525
Nitro-benzine manufacture, effect on blood ... ..	672
Parakeet, see Protozoal disease of	
Pigeons, see Ship-beri-beri	
<i>Piroplasma canis</i> ... ..	232
Plague bacillus, multiplication in flea's stomach ... ..	398
„ „ number in flea's stomach ... ..	397
„ „ in rectum and faeces of fleas ... ..	404
„ „ survival in fleas ... ..	406
„ „ virulence of, in plague rats ... ..	346, 465
„ bibliography ... ..	720
„ chronic, in rats ... ..	457
„ „ „ relation to epizootic ... ..	468
„ diagnosis of natural rat plague ... ..	324, 359
„ „ by cutaneous inoculation of animals etc. ... ..	346
„ in domestic animals other than rats ... ..	891
„ duration of infectivity of houses ... ..	885

Plague dwellings, <i>see</i> influence of	
" epidemics, experimental in animals ... ..	421
"       "       relation to epizootic ... ..	703, 746, 762, 764, 858
" epidemiology, digest of recent observations on ... ..	694
"       "       observations in Bombay City and Island ... ..	724, 874
" epizootic, <i>see</i> rat	
" experiments in Bombay plague houses ... ..	436
"       "       with caged animals in plague houses ... ..	979
"       " <i>see</i> flea, guinea-pig, rat	
" fleas, anatomy of ... ..	446
"       "       experimental infections through their agency ... ..	388, 411, 436, 977
"       "       infection through ... ..	415, 437
"       " <i>Pulex cheopis</i> , capacity of its stomach ... ..	395
"       "       "       external anatomy ... ..	446
"       "       "       natural occurrence on man ... ..	472
"       "       " <i>see</i> bacillus	
"       " <i>Pulex felis</i> and <i>P. irritans</i> experiments with ... ..	412 et seq., 881
"       "       on rats in Punjab villages ... ..	904, 914
"       "       in relation to plague ... ..	837
"       "       seasonal prevalence in Punjab villages ... ..	914
"       "       "       in relation to plague ... ..	440
"       "       "       transported in clothing ... ..	888
" in guinea-pigs, buboes, in experimentally infected ... ..	392, 393
"       "       "       in naturally infected ... ..	392
"       "       "       in plague houses ... ..	809 et seq., 976
"       "       "       running free in plague houses ... ..	437
"       "       " <i>see</i> experiments with caged	
" human cases in Kasel ... ..	940, 959
"       "       "       modes of infection ... ..	705, 875
"       "       "       direct contact ... ..	709
"       "       "       infected clothing, food etc. ... ..	712, 886
"       "       "       infectivity of houses ... ..	711, 758, 811, 896
"       "       "       importation of infection ... ..	713
"       "       "       influence of insanitary conditions ... ..	714, 768
"       "       "       seasonal prevalence ... ..	717
" human and rat cases in Dhand, Punjab ... ..	932, 946
" infection of animals through fleas, experiments ... ..	437 et seq.
"       "       of man, <i>see</i> human	
"       "       imported into uninfected places ... ..	889
"       "       mode of, in nature ... ..	700
"       "       of rats, feeding experiments ... ..	373
" influence of dwellings on, in Bombay city ... ..	768 et seq.
"       "       "       Punjab villages ... ..	811 et seq., 896, 899
"       "       "       of evacuating infected houses ... ..	957
"       "       "       habits of people ... ..	782
"       "       "       overcrowding ... ..	780
" in monkeys ... ..	892

	PAGE
Plague observations on, in Punjab villages (Dhand, Kasel) ... ..	895
"      "      relating especially to Dhand ... ..	918
"      "      "      "      "      Kasel ... ..	939
"      "      on, in villages, in Sion ... ..	801
"      "      "      "      "      Parel ... ..	843
"      "      "      "      "      Wadhala ... ..	840
"      "      "      "      "      Worli ... ..	866
"      pathological histology in rat ... ..	359
"      rat, abdominal organs, condition of ... ..	331, 368
"      "      breeding season ... ..	749, 905
"      "      buboes in ... ..	327, 378, 382 et seq.
"      "      chronic plague in ... ..	457, 719
"      "      collecting ... ..	735, 845
"      "      cutaneous inoculation of, with <i>B. pestis</i> ... ..	346 et seq.
"      "      diagnosis of plague in ... ..	324, 359
"      "      epizootic in relation to epidemic ... ..	421, 696, 702-3, 762-4, 822, 858, 867, 901, 922, 927, 932, 943, 953, 957
"      "      "      "      "      place ... ..	754, 758
"      "      "      origin of ... ..	926, 945
"      "      "      affecting <i>Mus decumanus</i> and <i>M. rattus</i> ... ..	758
"      "      "      seasonal prevalence ... ..	90, 745, 752
"      "      "      severity and extent ... ..	756, 927
"      "      "      species of rats affected ... ..	742
"      "      examination of for plague ... ..	736, 854
"      "      experimental infection, effects ... ..	368
"      "      feeding, experimental infection by ... ..	373, 380
"      "      and fleas in Punjab villages ... ..	901, 904, 912
"      "      general mortality in ... ..	908
"      "      in houses ... ..	437, 957
"      "      infection by feeding ... ..	373
"      "      migration of ... ..	907
"      "      natural history of, in relation to epizootic ... ..	703, 746, 904
"      "      not infected by urine from plague cases ... ..	380
"      "      plague-like diseases in ... ..	337
"      "      post-mortem appearances in ... ..	327 et seq., 368, 377
"      "      "      "      "      "      chronic plague ... ..	462
"      "      virulence of <i>B. pestis</i> in ... ..	346 et seq., 465
"      recurrence in given houses ... ..	984
"      rodents etc. affected ... ..	742, 760-1
"      spread of infection in houses ... ..	874, 882
"      "      "      "      "      hospitals ... ..	875
"      "      "      "      "      by imported cases ... ..	876
"      "      "      "      " <i>Pulex irritans</i> ... ..	881
"      "      "      "      "      septicaemic human cases ... ..	881
"      "      "      "      "      fleas, q.v. ... ..	
"      statistics of incidence on population ... ..	763, 794
"      summaries of sections ... ..	761, 767, 893

	PAGE
Plague transmission to rat by feeding ... ..	373
"                    "          "          etc. <i>see</i> flea	
"          urine of plague cases, fed to rats ... ..	380
"          ventilation, <i>see</i> influence of dwellings	
"          villages, <i>see</i> observations in	
" <i>see</i> "Cattle-Plague"	
Plague-like diseases in rats ... ..	337
Polyneuritis gallinarum ... ..	619, 634
Poultry, <i>see</i> Ship-beri-beri	
Protozoa, <i>see</i> <i>Piroplasma</i> , <i>Trypanosoma</i>	
Protozoal disease of muscles of Parakeet ... ..	552
Publications received ... ..	161, 686
<i>Pulex</i> , <i>see</i> under Plague flea	
Rat, <i>see</i> Plague	
Rheumatism, acute, <i>see</i> Meteorological	
Scarlatina, Streptococci in ... ..	599
" <i>see</i> Diphtheria	
Scurvy (experimental) ... ..	619, 634
Serum, <i>see</i> Antitoxin, Blood, Supersensitisation	
Serum-disease, <i>see</i> Supersensitisation	
"Ship-beri-beri" and Scurvy (experimental) ... ..	619, 634
Soil, behaviour of <i>B. coli</i> etc. in ... ..	494
<i>Spirochaeta pertenuis</i> , <i>see</i> Yaws	
Streptococci in scarlatina ... ..	599
Supersensitisation by serum injections ... ..	35, 61, 607
Syphilis, experimental, in monkey ... ..	561
Tetanus toxin and antitoxin compounds ... ..	101
Toxic compounds of tetanus toxin etc. ... ..	101
Toxicology of anilin dyes ... ..	672
"          "          carbon monoxide ... ..	528
"          "          nickel carbonyl ... ..	525
"          "          nitro-benzine ... ..	672
Toxin-antitoxin reaction ... ..	501
Toxin of <i>B. diphtheriae</i> , estimation of ... ..	512, 589
<i>Trypanosoma brucei</i> etc. colour-therapy against ... ..	281
" <i>dimorphon</i> , colour-therapy against ... ..	273
Vaccines for Malta fever ... ..	115
Vaccinia in animals, effect of light upon ... ..	155
Water, wells, bacteriological examination ... ..	479
"          "          fluorescein test for pollution of ... ..	182
Yaws, in monkeys ... ..	558
"          flies as transmitters ... ..	566



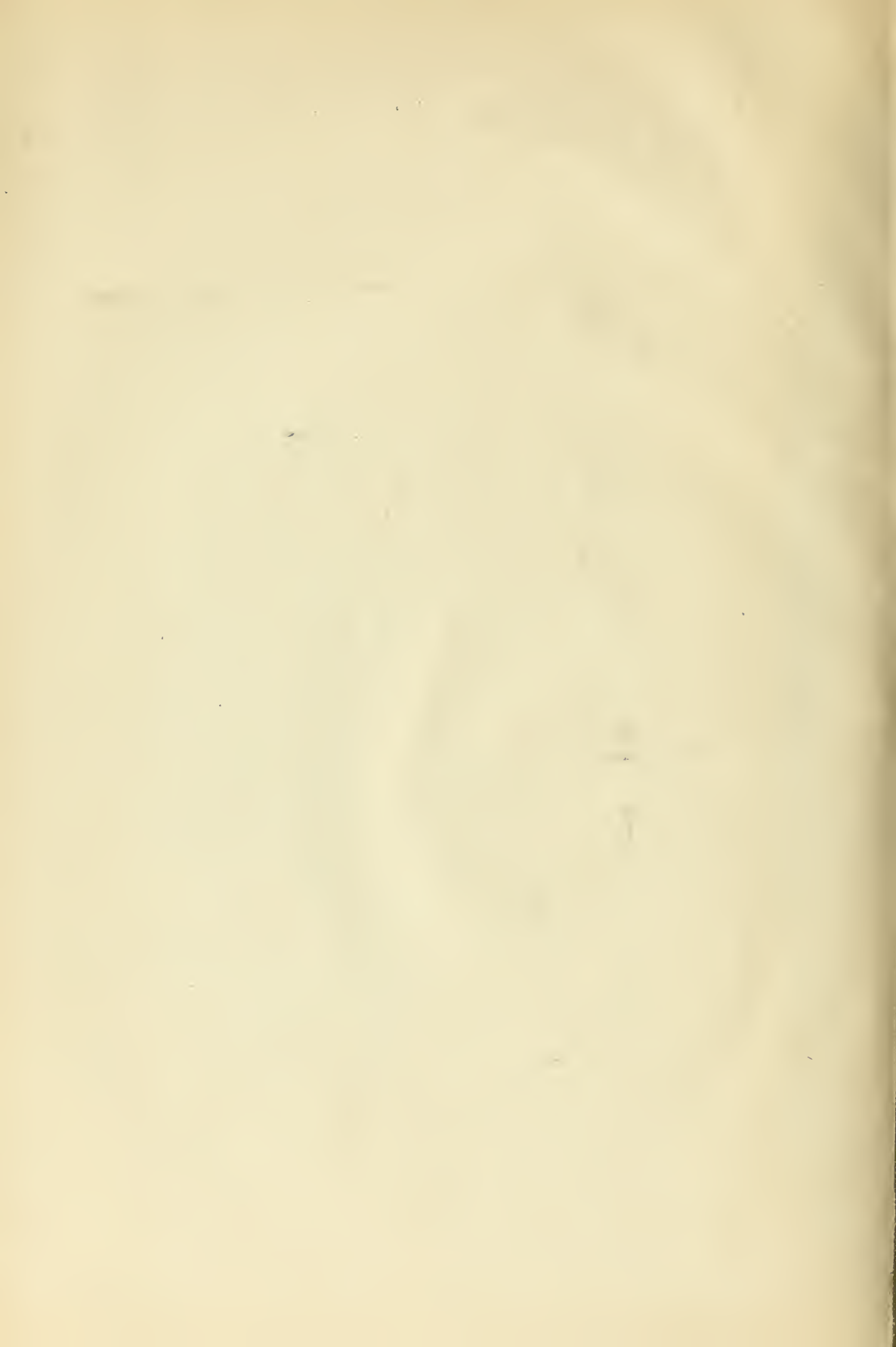














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